CLINICAL STUDY REPORT

ATROPHOGENIC POTENTIAL OF CALCIPOTRIOL, BETAMETHASONE AND MOMETASONE

A single-centre, prospective, randomised, double-blind, within-subject, comparative study of the atrophogenic potential of:

1. Calcipotriol ointment (50 µg/g),
2. Betamethasone 17-Valerate ointment (1 mg/g),
3. Mometasone Furoate ointment (1 mg/g), and
4. Vehicle of Calcipotriol ointment in Healthy Volunteers

The clinical study report has been redacted using the following principles: Where necessary, information is anonymised to protect the privacy of study subjects and named persons associated with the trial as well as to retain commercial confidential information. Summary data are included but data on individual study subjects, including data listings, are removed. This may result in page numbers not being consecutively numbered. Access to anonymised data on individual study subjects may be obtained upon approval of a research proposal by the Patient and Scientific Review Board. Appendices to the clinical study report are omitted. Further details and principles for anonymisation are available in the document LEOPHARMA PRINCIPLES FOR ANONYMISATION OF CLINICAL TRIAL DATA.

MCO 9407 CAN STUDY

Leo Pharma Inc. Final
Medical Department November 27, 2001
COMPLIANCE WITH GOOD CLINICAL PRACTICE

This Study Report is designed to comply with the Good Clinical Practice (GCP) standards issued by the International Conference on Harmonisation (ICH) (topic E 6 CPMP/ICH/135/95 and E 3 CPMP/ICH/137/95: Structure and Content of Clinical Study Reports.)
This study was performed in compliance with the Good Clinical Practice (GCP) standard issued by the International Conference on Harmonisation (ICH), the Declaration of Helsinki with subsequent amendments, and respecting national rules/regulations.

The study was performed in accordance with the approved Study Protocol and with LEO Pharma Standard Operating Procedures for GCP. The report provides a true and accurate record of the results obtained.

Authorised by: PCPC

Date 26/10/02

Distribution: Original → Trial Master File (as part of original Study Report)

Version: 01/05/02
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1 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

1.1 LIST OF ABBREVIATIONS

CRF  Case Report Form
ICH  International Conference on Harmonisation
IEC  Independent Ethics Committee
IRB  Institutional Review Board
GCP  Good Clinical Practice
Leo  Leo Pharmaceutical Products

1.2 DEFINITION OF TERMS

Adverse Drug Reaction (ADR)

In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. Regarding marketed medicinal products: a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).
Adverse Event (AE)
Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Atrophy
A reduction in skin thickness, primarily involving reduced dermal connective tissue layer prominence, but also involving epidermal flattening.

Audit
A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analysed and accurately reported according to the protocol, Sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

Concomitant Medication
Any medication taken by a subject apart from the Investigational Product(s).

Contract Research Organisation (CRO)
A person or an organisation (commercial, academic, or other) contracted by the Sponsor to perform one or more of a Sponsor's trial-related duties and functions.
Enrolled Patient
A patient who has signed an informed consent and been assigned a CRF number.

Fraud
Fabrication of data, selective and undisclosed rejection of undesired results, substitution with fictitious data, deliberately incorrect use of statistical methods for the purposes of reaching other conclusions than those warranted by the data, misinterpretation of results and conclusions, plagiarism of results or entire articles from other researchers, misrepresentation of other researchers' results, unwarranted authorship, and misleading application for positions or funds.

Good Clinical Practice (GCP)
A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected.

Informed Consent
A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.

Inspection
The act by a regulatory authority(ies) of conducting an official review of documents, facilities, records, and any other resources that are deemed
by the authority(ies) to be related to the clinical trial and that may be located at the site of the trial, at the Sponsor's and/or contract research organisation's (CRO's) facilities, or at other establishments deemed appropriate by the regulatory authority(ies).

**Investigational Product**
A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorisation when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.

**Investigator**
A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator. See also Subinvestigator.

**Investigator Trial File**
The collection of trial documents required by Leo SOPs, ICH Guidelines and/or regulatory requirements to be on file at the investigator site.

**Local Clinical Project Co-ordinator (LCPC)**
The person appointed by Leo to be Leo's national representative responsible for all aspects of a clinical trial within a country.

**Monitor**
A person appointed by the Sponsor to carry out monitoring of a clinical trial.
Monitoring
The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

Patient Screening Log
A document which identifies patients/subjects who entered pre-trial screening.

Principal Clinical Project Co-ordinator (PCPC)
The person appointed by Leo to be Leo’s main international representative responsible for all aspects of a clinical trial.

Quality Assurance (QA)
All those planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirement(s).

Randomisation
The process of assigning trial subjects to treatment or control groups using an element of chance to determine the assignments in order to reduce bias.

Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (S-ADR)
Any untoward medical occurrence that at any dose:
- results in death,
- is life-threatening,
- requires inpatient hospitalisation or prolongation of existing hospitalisation,
• results in persistent or significant disability/incapacity,
• is a congenital anomaly/birth defect,
or
• other medically important conditions.

Source Data
All information in original records and certified (dated and signed) copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source Documents
Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

Sponsor's Medical Expert
A medically qualified person appointed by the Sponsor to be responsible for medical questions relating to a trial.

Statistical Analysis Plan
A document that contains a more technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes detailed procedures for executing the statistical analysis of the primary and secondary variables and other data.
Striae
A visible, linear scar, commonly referred to as “stretch” marks, and associated with prolonged use of corticosteroids.

Subinvestigator
Any individual member of the clinical trial team designated and supervised by the investigator at a trial site to perform critical trial-related procedures and/or to make important trial-related decisions (e.g., associates, residents, research fellows). See also Investigator.

Telangectasia
A condition associated with prolonged use of corticosteroids, wherein small blood vessels become permanently dilated and increasingly visible through the skin.

Unexpected Adverse Drug Reaction (U-ADR)
An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator’s Brochure for an unapproved Investigational Product or package insert/summary of product characteristics for an approved product).
2 ABSTRACT

Objectives
The objectives of the study were to compare the atrophogenic potential of up to 6 weeks use of calcipotriol ointment, 50 μg/g, betamethasone 17-valerate ointment, 1 mg/g, mometasone furoate ointment, 1 mg/g, and the vehicle of calcipotriol ointment, in healthy volunteers.

Study Design
The study was a single-centre, prospective, randomised, double-blind, within-subject comparative study of the atrophogenic potential of:
1) Calcipotriol ointment (50 μg/g), BID
2) Betamethasone 17-valerate ointment (1 mg/g), BID
3) Mometasone furoate ointment (1 mg/g), OD, and vehicle ointment OD, and
4) Vehicle of calcipotriol ointment BID in healthy volunteers.

Methodology
Included were healthy volunteers of either sex and aged 18 years or older. Subjects must have given written informed consent, and females must have demonstrated not to be pregnant at the time of recruitment, and agreed not to become pregnant during the study. Excluded from the study were subjects with pre-existing atrophy, hypersensitivity to any of the investigational products or other skin diseases in the target areas of the abdomen.

At the first visit, the subjects were assessed for eligibility with respect to the inclusion and exclusion criteria. Female subjects had a urine pregnancy test at this visit. Eligible subjects were enrolled in the double-blind "treatment" phase, lasting 6 weeks. Subjects attended visits at 2, 4, and 6 weeks after being randomised. During the study, subjects
applied each of the four randomised treatments to one of four 4 cm x 4 cm areas of the abdomen. The treatment tubes were identically labelled, except that each indicated a different abdominal treatment area to which that particular treatment was applied and there were separate tubes marked for the morning and evening application. All treatments were applied twice daily, except in the mometasone furoate arm, wherein mometasone furoate was applied once daily plus vehicle ointment was applied once daily.

At baseline and after 2, 4 and 6 weeks, the presence of atrophy was determined by means of ultrasound examination, unaided visual examination and magnified visual examination. Reports of adverse events were elicited with a non-leading question at all on-treatment visits.

The Primary Response Criterion was the change in skin thickness, as measured by ultrasound, induced by each of the four treatments in the four treated areas of the body.

Results
The mean change in skin thickness from baseline to subsequent visits was not the same for all treatments (P=0.017). The mometasone treated sites had a far larger degree of skin thinning compared to the other treated sites. The mean change in skin thickness from baseline to each subsequent visit was similar for sites treated with betamethasone, calcipotriol and the vehicle of calcipotriol ointment. There was no statistically significant effect of time (P=0.85) and no evidence of any treatment by time interaction (P=0.91).

The skin thickness for the untreated site decreased with each subsequent visit, and had the greatest decrease in thickness of any site.
Adverse events judged possibly or probably due to use of study medication were reported by a total of 2 subjects, including:

1 subject given Betamethasone 17-valerate ointment (1 mg/g) BID
1 subject given Mometasone furoate ointment (1 mg/g) OD and vehicle ointment OD

“Folliculitis” was reported by 1 subject on a total of 1 treatment location, and “Rash erythematous” was reported by 3 subjects on a total of 2 treatment locations.

No withdrawals due to adverse events which were judged possibly/probably related to use of the study drugs occurred. One subject was withdrawn following an appendectomy at week 3. This subject was replaced.

Conclusion

The application of corticosteroid ointments induced a thinning of the skin that was either significantly greater than that of calcipotriol (mometasone furoate applied once daily), or showed a trend to exceed the effect of calcipotriol (betamethasone valerate applied twice daily). However because of the difficulties in interpreting the data from the ‘control (untreated) site’ the results of the study cannot be considered reliable.
**CLINICAL STUDY REPORT APPROVAL FORM**

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<td>Atrophogenic potential of calcipotriol, betamethasone and mometasone</td>
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By signing below I confirm that I have read the Clinical Study Report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

**APPROVAL BY INTERNATIONAL CO-ORDINATING INVESTIGATOR**

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**APPROVAL BY HEAD OF DERMATOLOGICAL MEDICAL DEPARTMENT, LEO DENMARK**

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**APPROVAL BY HEAD OF MATHEMATICAL-STATISTICAL DEPARTMENT, LEO DENMARK**

(or BIOMETRIC DEPARTMENT in UK)

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**Distribution:**
- Original → Trial Master File
- Copy → Study Report

**Version:** 01/05/01
4 REPORT AUTHORS

[Redacted] BSc., DVM
Medical Department
Leo Pharma Inc.
555 Kingston Road West
Ajax, Ontario, CANADA
L1S 6M1
Tel: [Redacted]
Fax: [Redacted]
e-mail: [Redacted]

[Redacted] BSc MSc
Medical Department
Leo Pharmaceuticals
Longwick Road
Princes Risborough
Bucks HP27 9RR
UNITED KINGDOM
Tel: [Redacted]
Fax: [Redacted]
e-mail: [Redacted]
5 INVESTIGATORS AND STUDY SITES

CANADA (6 Investigators at 1 Centre)

[Name], MD, FRCPC, FACP

Tel: [Contact Information]

[Name], MD, FRCPC, FACP

Tel: [Contact Information]

[Name], MD, FRCPP

Tel: [Contact Information]

[Name], MD, FRCPC, FMSQ

Tel: [Contact Information]

Ultrasound measurements:

[Name], Ph.D.

Tel: [Contact Information]
COMPANY PERSONNEL

PCPC, LCPC

[Redacted], MBCh B

Medical Department
Leo Pharma Inc.
555 Kingston Road West
Ajax, Ontario, CANADA
L1S 6M1

STATISTICIAN

[Redacted] BSc. MSc.

Medical Department
Leo Pharmaceuticals
Longwick Road
Princes Risborough
Bucks HP27 9RR
UNITED KINGDOM
7 ETHICS

7.1 ETHICAL CONDUCT OF THE STUDY

The study was conducted to conform with the principles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly, 1964, and subsequent amendments.

The protocol was approved by/received favourable opinion from the relevant Institutional Review Board (IRB) prior to inclusion of subjects.

The patient's signed and dated informed consent to participate in the study was obtained prior to any study related procedure being carried out. If the patient was unable to read or consent, the procedures to be applied were described in detail and approved by the IRB.

The study was approved/notified by the appropriate Regulatory Authority as required.

The study was conducted in accordance with the principles of GCP.

7.2 ETHICAL CONSIDERATION STATEMENT

While effective in the treatment of psoriasis, corticosteroid preparations are known to cause skin atrophy when used in topical preparations. Alternative, equally-as-elegant topical preparations with a similar degree of anti-psoriatic efficacy would be an advance in therapy. Under controlled conditions, this study compared the effects of low quantities of such medications used in non-visible (under clothes) body areas of healthy volunteers. Study subjects were followed by dermatologists, and were not considered at any undue risk during the trial. Subjects could be withdrawn at any time during the study, and were followed at frequent intervals (every 2 weeks) during the course of the trial. All
data recorded was coded and did not identify any persons taking part in
the trial, in accordance with the EU Data Protection Directive
(95/46/EF).

7.3 PATIENT INFORMATION AND INFORMED CONSENT

All subjects received written and verbal information concerning the
study. This information emphasised that participation in the study was
voluntary and that the subject could have withdrawn from the study at
any time and for any reason. All patients were given opportunity to ask
questions and were given sufficient time to consider before consenting.

All Investigators signed an "Investigator's Agreement" outlining the
above.

7.4 INSURANCE AND LIABILITY

The patients in the present study were covered by the product and
general liability insurance of Leo Pharmaceutical Products in the event
of trial-related injury or death, in accordance with applicable law and
with the CPMP Note for Guidance on Good Clinical Practice
(CPMP/ICH/135/95) of 17 July 1996.
8 INTRODUCTION AND RATIONALE

8.1 SKIN ATROPHY

Skin atrophy is a well-recognized risk of topical corticosteroid therapy (1). This condition predisposes subjects to injury, is cosmetically unacceptable and increases the overall cost of therapy for a given initial condition, since additional or alternative treatment must often be prescribed.

Skin atrophy can be detected visually by changes in the appearance of the skin. Atrophied skin becomes thin and fragile, and takes on a translucent appearance. Telangiectasia, a condition wherein small blood vessels become permanently dilated and increasingly visible through the skin, is seen in atrophic skin due to many etiologies, including use of corticosteroids. The appearance of striae can also be associated with use of topical/prolonged treatment with corticosteroids. Striae are visible linear scars commonly referred to as "stretch marks", and indicate an area of dermal damage caused by dermal stretching. They can be associated with many other causes, including pregnancy, obesity, rapid growth, and Cushing's syndrome (2, 3).

The risk of skin atrophy increases with increasing potency of a corticosteroid and with increasing duration of therapy (4, 5). The effectiveness of the stratum corneum barrier also determines the degree of atrophy experienced for a given treatment (5). As such, areas of the body where penetration of the steroid is greatest, including the face, genitals, areas of folds where there is natural occlusion (axilla, groins; under breasts) and in the young and elderly in general, will be more prone to experience steroid-induced atrophy.
Corticosteroid-induced skin atrophy is thought to be primarily due to the action of corticosteroids on connective tissue. Connective tissue is comprised of various cell types, including fibroblasts, mast cells and histiocytes (macro- and monocytes), a matrix material and protein fibres, including primarily collagen and elastin.

Fibroblasts are the most numerous cell found in the loose connective tissue in the body (e.g., in the skin), and can transform into several types of specialist cells, including myofibroblasts, smooth muscle cells, chondrocytes and osteocytes. There are conflicting views as to whether the different phenotypes arise from pluripotent stem cells, or from subtypes with restricted properties of differentiation. The cell has well developed endoplasmic reticulum, and Golgi structures, and prominent ribosomes - all characteristic of actively synthesizing and secreting cells.

Most researchers are in agreement that fibroblasts are the source of the ground substance/matrix of connective tissue (6). The matrix material is made of proteoglycans, a family of macromolecules formed by the joining of a core protein to various unbranched disaccharide units called glycosaminoglycans (GAGs). The proteoglycan/GAG complexes are hygroscopic macromolecules which have a large capacity to attract and bind water molecules.

Running through the matrix are various protein fibres, of which interstitial collagen and elastin are the most common. Fibroblasts have been shown to produce collagen in vitro, and shown to produce at least collagen precursors in vivo. Collagen makes up 75% of the dry weight of the dermal connective tissue, and occupies about 18 - 30% of the dermal volume. The term “Collagen” describes a family of strong, soft, flexible but inelastic proteins, numbered from Collagen type 1 through to (at least) Collagen type 20. Of these, type 1 is the most common in the
dermis (70%), with type III making up a further 15% of the dermal collagen.

In fibroblasts, glucocorticoid receptor binding results in a decrease in the amount of collagen-mRNA, either by lowering the rate of transcription or by increasing the rate of mRNA degradation (7). The fibroblasts themselves become shrunken, but their numbers do not decrease. Corticosteroids also decrease the activities of enzymes responsible for the synthesis of collagen, most notably prolyl hydroxylase (8). In addition, the production of glycosaminoglycan (GAG) by fibroblasts is reduced. As GAG has marked water-retaining properties, a rapid reduction in dermal thickness is observed as GAG levels decrease (5).

Corticosteroids also cause a marked thinning of the epidermis, with flattening of the rete ridges and decreased corneocyte size (9). The mechanism of this change is not known for certain, but is thought to be related to decreased mitotic activity in the basal stratum of the epidermis.

The vasoconstrictor effect of corticosteroids, which affects the superficial capillaries of the dermis, may induce atrophy through prolonged ischemia of the treated area (10).

Corticosteroid-induced atrophy may be transient or permanent (11). Optimal use of corticosteroids (i.e., using the lowest potency possible for the shortest period of time) or discontinuous application may decrease or eliminate the chance of permanent atrophy (12). It has been shown, however, that even discontinuous use of higher potency corticosteroids can lead to irreversible atrophy (11).
A number of quantitative methods have been used to measure skin thickness. These have included radiographic methods, the use of skin callipers and direct measurements of skin biopsy mounts.

Ultrasonography has been used extensively as a means to measure skin structure and thickness (13). Several studies using this technique to examine the atrophogenic effects of corticosteroids have been published. Ultrasound measurements have been shown to correlate well with histological measurements of skin thickness, and have the advantage of being non-invasive (14). In addition to permitting the measurement of skin thickness, some structural analysis is also possible by examining the echo-density of the skin on "B-mode" scanning. The measurement of these parameters has been facilitated by the development of computer-assisted image analysis.

Double-ester corticosteroids, such as mometasone furoate, have been developed in an attempt to produce a corticosteroid with a lower atrophogenic propensity than other corticosteroid forms. There is, however, evidence that even these compounds may produce atrophy.

Regarding topical treatment of psoriasis, a treatment that offers equal efficacy and cosmetic acceptability as steroids, with less or no atrophogenic potential, would clearly represent an advance in therapy.

In the large, randomized clinical trials completed to date with calcipotriol, each on-treatment visit has involved both subjects and investigators observing the subjects for adverse effects, but atrophy has not been specifically measured as a safety parameter. In over 10,000 trial visits to date, no calcipotriol-induced atrophy has been noted.

Smaller trials, however, have addressed the issue of atrophy. In an open, non-comparative trial, 15 subjects who had completed an
ointment dose-ranging study (16) were subsequently treated in another trial for an average of 31 weeks (range 15 - 41 weeks) with twice daily calcipotriol ointment (50 μg/g). Skin biopsies taken before and at the end of calcipotriol treatment showed no evidence of skin atrophy having developed during long-term treatment with calcipotriol ointment (17).

A second study compared the atrophogenic effect of calcipotriol ointment to clobetasol ointment, prednicarbate ointment and the vehicle of calcipotriol ointment (18). All treatments were applied under occlusion and skin thickness was measured using ultrasonography. Both corticosteroid ointments resulted in skin atrophy, whereas no atrophy was noted with the vehicle treatment. The calcipotriol treatment resulted in a thickening of treated skin areas, attributed to an irritant dermatitis.

8.2 CALCIPOTRIOL

8.2.1 Pharmacology

The vitamin D analogue “calcipotriol” was synthesised at the research laboratories of Leo Pharmaceutical Products. Pre-clinical studies demonstrated calcipotriol to have a high binding affinity to the cellular receptor for the biologically active form of vitamin D₃ (19). On a molar basis, it was equi-potent to calcitriol in terms of suppressing cellular proliferation and promoting cellular differentiation in various cell lines, including keratinocytes. What made calcipotriol particularly desirable in terms of pursuing it in further clinical studies was its very small systemic effect on calcium metabolism in rats - about 100 to 200 times less than that of calcitriol (19). This is now primarily ascribed to the very short half life of calcipotriol (about 11 minutes), which in turn makes it an ideal topical agent for treating skin diseases.
8.2.2 Overall Development of Calcipotriol Topical Products

Clinical studies of calcipotriol started in 1986. Initial work employed "first generation" creams, containing 33 μg/g and 100 μg/g of calcipotriol. When applied twice daily for 6 weeks to psoriatic lesions, the creams were shown to be significantly better than placebo in terms of reducing erythema, thickness and scaliness in patients with psoriasis vulgaris (20). No adverse events were reported and laboratory testing showed no significant changes in any parameter that was monitored. It was especially noteworthy that no change in serum ionised calcium was found.

In an effort to improve efficacy, calcipotriol was dissolved in an ointment base, then used in a dose finding trial to treat psoriasis patients. In this trial (21) an ointment containing 50 μg/g was deemed the optimal concentration for future trials in psoriasis.

Subsequently, a very large clinical trial program was carried out, leading to the development of both ointment and cream formulations for treatment of non-triginous body areas, and a solution formulation for scalp psoriasis. This was the most extensive, well-documented clinical trial program involving a topical antipsoriatic agent ever conducted.

In virtually all studies, the efficacy of the product was examined using a modified PASI (Psoriasis Area and Severity Index) (22) system. It is worth recognising that this assessment tool measures the impact of treatment on the patient's body psoriasis as a whole, rather than the effect of treatment on a particular lesion. When assessing the efficacy of topical therapy, this methodology provides a conservative figure, in that improvement of one treated lesion can be negated in terms of the PASI score should another lesion develop in an untreated area. Additionally,
investigators and patients provided their “overall” estimation of the treatment effect.

The safety of the drug was followed through measuring the impact on various serum biochemistry parameters, particularly those involved in calcium homeostasis, and liver and renal function. Some studies also measured the effect of treatment on urine biochemistry. Adverse event reports were solicited in a non-leading manner at all on-treatment study visits.

These studies demonstrated that:

1) calcipotriol was an effective antipsoriatic agent when used in short treatment periods lasting up to 8 weeks (20,21,23-25).
2) calcipotriol had a rapid onset of action, with the first 2-4 weeks of treatment showing the greatest rate of improvement (21,22,23-25).
3) calcipotriol, used as directed for the treatment of mild to moderate psoriasis, was a safe treatment when used to treat psoriasis for up to 8 weeks per treatment course. Serum calcium, liver and renal parameters, urinary calcium and skin biopsies failed to show deleterious effects of treatment (20,26-29).
4) calcipotriol was effective for the long term control of psoriasis (30-32).
5) calcipotriol was safe when used for the long term control of psoriasis (30-32).
6) calcipotriol could be combined with other modalities of treatment to increase efficacy and/or increase the safety of antipsoriatic therapy, and/or treat more severe forms of the disease (33-41,46).
7) calcipotriol was a cost-effective agent (42).
8.2.3 Studies with Ointment

Over 25 studies with the ointment formulation of calcipotriol, involving over 3,500 psoriasis patients, have been conducted in the clinical trial program.

The studies conducted with the ointment formulation have been designed to show:

1. the ideal concentration of calcipotriol ointment.
2. the absorption profile of the ointment.
3. the effect on calcium homeostasis of the ointment.
4. comparative efficacy and safety of the calcipotriol ointment to vehicle.
5. comparative efficacy and safety of the calcipotriol ointment to other registered, single agents.
6. comparative efficacy and safety of combinations of calcipotriol ointment and other treatment modalities to various comparators.
7. efficacy and safety in special populations (children).

These studies have demonstrated equal or superior efficacy to other single, registered agents, including Dithranol, tar preparations, and medium and high potency topical corticosteroid formulations, as follows:
Other studies have explored the benefits of calcipotriol when used in combination with other antipsoriatic agents. Generally, the addition of calcipotriol to more toxic agents lessened the dose of the more toxic agent with no loss in and/or an increase in efficacy.
### 8.3 RATIONALE

This trial compared the relative atrophogenic properties of non-occluded use of marketed preparation of ointments containing calcipotriol, betamethasone 17-valerate, mometasone furoate and the vehicle of calcipotriol ointment. The list of comparators was and is relevant, in that it involves the most commonly prescribed topical treatment for psoriasis, betamethasone-17-valerate, and mometasone furoate, a steroid whose structure is intended, at least in part, to minimise the risk of atrophy.

The observations for atrophy were made on treated areas of “normal” abdominal skin. Both visual and ultrasonographic methods were employed in making this assessment. Abdominal skin, which has been
shown to be equally as prone to corticosteroid-induced atrophy as other areas (47), was chosen as the treatment area for the following reasons:

1) for ease of application of study medication (the back would not be appropriate as it would be difficult for the subject to apply the treatment for him or herself, and
2) for ease of measurement of atrophy, and
3) to confine any atrophy which may result from any of the treatments to cosmetically acceptable regions (hidden under clothing).
9 INVESTIGATIONAL PLAN

The full protocol is presented in Appendix IV.

9.1 STUDY OBJECTIVE

To determine whether there is a difference between the atrophogenic properties of calcipotriol ointment (50μg/g), betamethasone 17-valerate ointment (1mg/g), mometasone furoate ointment (1mg/g) and the vehicle of calcipotriol ointment in normal skin of healthy volunteers.

9.2 STUDY DESIGN

The study was a single-centre, prospective, randomised, double-blind, within-subject comparative study of the atrophogenic potential of the following 4 treatments:

1) Calcipotriol ointment (50 μg/g) twice daily.
2) Betamethasone 17-valerate ointment (1 mg/g) twice daily.
3) Mometasone furoate ointment (1 mg/g) once daily and the ointment vehicle of Mometasone furoate once daily
   and
4) Vehicle of calcipotriol ointment twice daily in healthy volunteers.

Eligible subjects were treated for up to 6 weeks. Subjects attended visits at 2, 4, and 6 weeks after being randomized. During the study, subjects applied each of the four randomized treatments to one of four 4 cm x 4 cm areas of the abdomen, as defined by a template. The ointments were applied twice daily without occlusion. The presence of atrophy was determined by means of ultrasound examination, unaided visual examination and magnified visual examination. These assessments
were made on each of the 4 treatment areas, and also on an area of untreated skin, also defined by the template. Reports of adverse events were elicited with a non-leading question at all on-treatment visits.

<table>
<thead>
<tr>
<th>Double-blind treatment</th>
<th>Week no.</th>
<th>Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 2 4 6</td>
<td>1 2 3 4</td>
</tr>
</tbody>
</table>

9.3 SUBJECT NUMBERS

A study comparing the atrophy which resulted from treatment of abdominal skin with amcinonide (47) showed thinning from an initial thickness of 1.73 mm to 1.29 mm in 22 days. The inter-subject standard deviations for these measurements was approximately 0.25 mm. Assuming that the intra-subject variation is at most the same as the inter-subject variation level, then if 15 subjects were enrolled, it was calculated that this would allow detection of a difference in thinning of 0.25 mm (15 per cent) between calcipotriol and the two steroids. All subjects would apply all four treatments simultaneously. The treatments would be randomised according to a 4 x 4 latin square design. In order to maintain balance with respect to the randomization, a total of 16 subjects were to be enrolled. Subjects who left the study were to be replaced and the replacement was to have received the same series of treatments as the subject who left the study.
9.4 CRITERIA FOR SUBJECT SELECTION

9.4.1 Inclusion Criteria

9.4.1.1 Subjects judged to be in generally good health.

9.4.1.2 18 years of age or older.

9.4.1.3 Either sex.

9.4.1.4 Signed informed consent had to be obtained from subjects after receiving verbal and written information about the study.

9.4.1.5 Females of child bearing ability had to have a negative pregnancy test and be using adequate contraception.

9.4.2 Exclusion Criteria

9.4.2.1 Subjects with hypersensitivity (defined as any history of suspected, significant intolerance OR as a contraindication) to the use of components of any of the study treatments (see Section 9.9).

9.4.2.2 The requirement for treatment during the study period, with any other medication (topical or systemic) that is associated with skin atrophy e.g., systemic or topical corticosteroids, retinoids, etc.

9.4.2.3 The presence of skin atrophy in the target areas, defined as an unaided visual assessment score greater than zero at the baseline visit.

9.4.2.4 Presence of "stretch marks" (striae atrophicae, striae distensae or lineae atrophicae) on the anterior abdomen, or a history of pregnancy, or excessive overweight (> 15 kg).
9.4.2.5 Subjects known or suspected of being unable to comply with the study protocol.

9.4.2.6 Subjects who were concurrently participating in any other clinical trial.

9.4.2.7 Subjects who had taken any investigational drug in the 3 months prior to Visit 1 (i.e., any agent whose active ingredient is not approved for sale in Canada).

9.4.2.8 Previous randomization in this study.

9.5 CRITERIA FOR EARLY WITHDRAWAL FROM THE STUDY

Subjects could have been withdrawn for any of the following reasons:

9.5.1 Voluntary Withdrawal: Subjects were free to withdraw from the study at any time and for any reason.

9.5.2 Medical Deterioration: The investigator was free to withdraw the subject at any time for medical reasons.

9.5.3 Adverse Events: Any adverse event that the investigator considered unacceptable to the subject.

9.5.4 Exclusion Criteria: Any exclusion criteria that became apparent during the trial.

9.6 TREATMENT ASSIGNMENT

At visit 1, subjects were assigned a subject ID number, corresponding to a specific CRF book number.
Based on their temporal order of presentation at the investigator's office for visit 1, subjects who satisfied all inclusion/exclusion criteria were assigned the next (ascending) randomisation number available at the centre. The randomisation number determined which of the four treatment areas was assigned each of the different topical medications. Randomisation was according to a computer generated random numbers table.

9.7 BLINDING OF STUDY

The betamethasone, mometasone and vehicle ointments were similar in appearance, smell and texture to calcipotriol ointment. Therefore, it was not considered possible to differentiate one treatment from another solely by sensory evaluation. All medications were supplied in identical packaging, and had tube-labelling that did not reveal the identity of their contents.

Mometasone furoate was used only once daily, whereas the other treatments were to be used twice daily. In order to preserve the masking of the study, all subjects were given separate tubes of each of the study treatments for morning and evening application. In the case of mometasone, only one of the tubes (morning) contained the active ingredient, and the other contained an unmedicated vehicle ointment identical in appearance to the mometasone furoate ointment.

9.8 BREAKING OF THE BLINDING

The investigator held a copy of the code for each individual subject in a separate, sealed envelope. The envelopes carried the subjects' randomization code number on the outside and the medication identification code on the inside.
The code was only to have been broken in an emergency wherein drug identification was necessary. In such an event, details of why the envelope was opened were recorded.

At the end of the study, all envelopes were collected by Trial Monitors.

9.9 INVESTIGATIONAL PRODUCTS

9.9.1 Calcipotriol

Calcipotriol ointment (Dovonex®), produced and certified by Leo Pharmaceutical Products, Ballerup, Denmark, contained 50 µg/g of calcipotriol, and the following vehicle excipients:

- Disodium hydrogen phosphate
- POE steareylether
- Propylene glycol
- Tetracemine disodium
- DL-alpha-tocopherol
- Petrolatum
- Paraffin liquid
- Purified water

Calcipotriol ointment was supplied in 30g tubes.

Batch # 9610602
9.9.2 Betamethasone 17-Valerate Ointment

Betamethasone 17-valerate (Betnovate®) ointment, produced by Leo Pharmaceutical Products, Ballerup, Denmark, contained 1 mg/g of betamethasone 17-valerate, and the following vehicle excipients:

- Liquid paraffin
- White soft paraffin

Betamethasone valerate ointment was supplied in 30g tubes.
Batch # 9615031

9.9.3 Mometasone Furoate

Mometasone furoate (Elocom® ointment), produced by Schering, contained 1mg/g of mometasone furoate and the following vehicle excipients:

- Hexylene glycol
- Propylene glycol stearate
- White petrolatum
- White wax
- Phosphoric acid to adjust pH
- Purified water

Mometasone furoate ointment was supplied in 30g tubes.
Batch # 9614631

Note: where applied, this was used in the morning, and the vehicle described in 9.9.4 was used in the afternoon.
9.9.4 Vehicle Ointment - Mometasone Furoate

A vehicle ointment, similar to the vehicle of mometasone furoate ointment and with ingredients similar to those listed in section 9.9.3, was used.

Vehicle ointment was supplied in 30g tubes.
Batch # 9614211

Note: where applied, this was used in the afternoon, and the ointment described in 9.9.3 was used in the morning.

9.9.5 Vehicle of Calcipotriol Ointment

Vehicle ointment similar in appearance, smell and texture to calcipotriol ointment, produced and certified by Leo Pharmaceutical Products, Ballerup, Denmark. The constituents of the vehicle ointment are listed in Section 9.9.1.

Vehicle ointment was supplied in 30g tubes.
Batch # 9610501

9.9.6 No Treatment

On each subject, an area of no treatment was assessed at each visit. This area was defined by the template described in Section 9.13.2.1.

9.9.7 Storage of Trial Medication

The trial medication was stored at room temperature (15 to 25°C) in a secure place.
9.10 ADMINISTRATION OF STUDY MEDICATION

Study medication was applied to four horizontally-aligned target areas of abdominal skin. Each target area measured approximately 4 x 4 cm. Subjects were provided with a plastic template which had four cutouts (designated A to D), one for each treatment area. The template had a small hole cut in it, to allow consistent placement with respect to the umbilicus, and an arrow to ensure proper vertical placement with respect to the sternum. Subjects were advised to use the templates in a standing position, and were advised to mark the treatment area daily with a felt pen.

Approximately 0.5 cm (length of medication as expressed from tube) of the 4 study treatments were evenly applied to one each of the four target areas, as designated on the tube label. The treatments were spread evenly in a thin layer over the treatment area and lightly rubbed in. All treatments were applied twice daily (at approximately 12 hour intervals) using a vinyl or latex finger protector to prevent both atrophy of hand skin and the spread of study treatments to other treatment areas. A separate protector was provided for each tube dispensed. No occlusion of target areas was permitted. Subjects were instructed to use the finger protector for that particular medication only and to wait 5-10 minutes after application before dressing.

9.11 DRUG ACCOUNTABILITY AND COMPLIANCE CHECKS

The investigators and their staff were fully responsible for maintaining adequate control of the test materials and for documenting all transactions with them. Trial medications were stored in a safe and secure place, and all “transactions” were recorded/documented.

All the trial medication supplied by and returned to Leo Laboratories were fully documented by the trial monitor and by the investigator.
Each subject was assigned their own box of study drug, identified on the outer box by the subject's unique randomisation number. Within the outer large box, study drug for dispensing at visits 1, 2 and 3 was packed in separate, smaller visit boxes. Within each visit box were distinct supplies of study drug for use on designated areas.

An inventory was kept of all other supplies issued by the company to the centre. All drug and other supplies returned to the company by the centre were reconciled with this inventory.

An inventory was kept of all study drug issued by the centre to each subject. Subjects were asked to return all used and unused tubes of trial medication at each subsequent visit. These tubes were stored by the Investigator until collected by the Trial Monitor.

9.12 CONCURRENT TREATMENT

Topical and systemic concurrent medication (except for agents mentioned in the exclusion criteria) were to be continued throughout the study, without change in dosage, wherever possible. Use of concurrent medication was recorded.
9.13 STUDY PROCEDURES

The following diagram summarises the study procedures:

<table>
<thead>
<tr>
<th>VISITS</th>
<th>Active Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEEKS</td>
<td>0 2 4 6</td>
</tr>
<tr>
<td>Written Informed Consent</td>
<td>*</td>
</tr>
<tr>
<td>Atrophy Assessments</td>
<td>*</td>
</tr>
<tr>
<td>Visual Exams</td>
<td>*</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>*</td>
</tr>
<tr>
<td>Recording of Adverse Events</td>
<td>*</td>
</tr>
<tr>
<td>Pregnancy test (6)</td>
<td>*</td>
</tr>
<tr>
<td>Supply of test medication</td>
<td>*</td>
</tr>
<tr>
<td>Collection of used test medication</td>
<td>*</td>
</tr>
</tbody>
</table>

1) In female subjects of child-bearing ability.

9.13.1 Medical History and Informed Consent

At visit 1, the subject's suitability for the study was checked using the inclusion and exclusion criteria. The subject's race, concurrent medication and current medical diagnoses, if any, was recorded. The subject's skin type was recorded as follows.

Skin type I - Always burn, never tan
Skin type II - Usually burn, tan less than average (with difficulty).
Skin type III - Sometimes burn, tan about average
Skin type IV - Rarely burn, tan more than average (with ease).
Skin type V - Moderately pigmented
Skin type VI - Heavily pigmented

The subject's signed informed consent to participate was obtained at visit 1.
9.13.2 Clinical Assessments - Atrophy

9.13.2.1 Ultrasound Assessments
At each visit, the target areas of abdominal skin were assessed using high frequency ultrasound. The skin was scanned in the B-mode using a 40 MHz or other suitable transducer. The transducer chosen could provide good resolution at depths up to approximately 2.5 mm. The same frequency transducer was used for all ultrasound images for the duration of the study.

Each of the four treatment areas, as defined in Section 9.10, was scanned at each of visits 1 (pre-treatment values), 2, 3, and 4. In addition, an area of untreated skin was also scanned at each of these visits.

The ultrasound technician was provided with a template similar to that used by the subject to guide treatment, but with smaller “target-area cut-outs”, to assist in guiding the ultrasound examination. The perforations were aligned to the centre of each treatment area, and there was an additional perforation to guide the imaging of an untreated area of skin. An image of the approximate centre point of each area was taken, followed by an image 0.5 cm to the right and left of the centre point. The final measured thickness was calculated as the mean of those repeated measurements shown to be perpendicular to the skin.

A hard copy of all ultrasound images was retained in the study archive.

9.13.2.2 Unaided visual assessment
At each visit, the target areas of abdominal skin were observed for signs of atrophy, based on a 5-point grading system as follows.

0 No difference compared with the surrounding skin.
1 Slight thinning, slight smoothing of the surface relief and only
just visible increase in transparency.

2 Moderate thinning, flattening of furrows and grooves, visible increase in skin transparency.

3 Severe thinning of epidermis, relief markedly reduced, increase in skin transparency.

4 Very severe thinning of epidermis with completely smoothed relief and very transparent skin. Clearly visible vasculature.

Photographs of each of the four target areas of abdominal skin were taken at each visit after visit 1. The images were used to confirm the results of the unaided visual assessment at the end of the study, and during routine monitoring of the study.

9.13.2.3 Magnified visual assessment of vascular architecture

At every visit, the target areas of abdominal skin were observed under oil/cover slip at 10X magnification. The increased/decreased ability of the investigator to observe the skin vasculature was recorded on a 5-point grading system described by Frosch et al (17):

0 No difference compared with surrounding untreated skin.
1 Capillary hyperemia with weak elongation and dilatation of a few vessels. Not visible with the naked eye.
2 Moderate telangiectasia, visible with the naked eye.
3 Clear telangiectasia with marked reduction of capillary loops.
4 Very advanced telangiectasia with complete absence of capillary loops.

9.14 CRITERIA FOR EFFICACY AND SAFETY

The four treatment groups were compared according to the following. The primary response criterion was the:
The change in skin thickness, as measured by ultrasound, in the areas of abdominal skin treated with each of the four treatments, from baseline to each subsequent visit.

The secondary response criteria were:
The change in skin thickness, as measured by ultrasound, from baseline to end of treatment.
The unaided visual examination at each visit and end of treatment.
The magnified visual examination at each visit and end of treatment.

9.15 ADVERSE EVENTS

At all visits after treatment start, the subject was asked a non-leading question by the investigator such as: “Since I last saw you, have you had any problems?”.

No specific symptoms were asked for.

If the answer was “NO”, no further questions were asked. If the answer was “YES”, the investigator recorded the event’s nature, intensity, duration, location, suspected causal relationship to the Investigational Product and outcome.

The investigator also observed the subject for any changes not reported by the subject, and recorded these changes.

9.15.1 Definition of (Serious) Adverse Events

Adverse Event (AE): any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding).
symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Adverse Drug Reaction (ADR): in the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Regarding marketed medicinal products: a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or modification of physiological function (see the ICH Guideline for Clinical Safety Data Management Definitions and Standards for Expedited Reporting).

Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (Serious ADR): any untoward medical occurrence that at any dose:
• results in death,
• is life-threatening,
• requires inpatient hospitalisation or prolongation of existing hospitalisation,
• results in persistent or significant disability/incapacity or
• is a congenital anomaly/birth defect, and
• other medically important condition.
(see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).
9.15.2 Recording of Adverse Events

Events either reported by the subject, or observed by the investigator, that fell into any of the above definitions were to be recorded on the adverse event page of the CRF book in the following manner:

The NATURE of the event was described in precise, standard medical terminology (i.e., not necessarily the exact words used by the subject). If known, a specific diagnosis was stated (e.g., allergic contact dermatitis).

The INTENSITY of the event was described in terms of mild, moderate or severe according to the investigator's clinical judgement.

- **mild.** The adverse event did not interfere in a significant manner with the subject's normal functioning level. It may have been an annoyance.
- **moderate.** The adverse event produced some impairment of functioning but was not hazardous to health. It was uncomfortable and/or an embarrassment.
- **severe.** The adverse event produced significant impairment of functioning or incapacitation and was a hazard to the subject.

The DURATION of the event was described by the start date and end date.

The CAUSAL RELATIONSHIP of the event to the use of the Investigational Product was described in terms of:

**Probable:** the adverse event
- followed a reasonable temporal sequence from administration of the Investigational Product
- could not be reasonably explained by the subjects clinical state, environmental or toxic factors or other therapies administered to the subject
• disappeared or decreased on cessation or reduction in dose of the Investigational Product
• followed a known pattern of response to the Investigational Product
• reappeared or worsened upon rechallenge.

Possible: the adverse event
• followed a reasonable temporal sequence from administration of the Investigational Product
• could have been reasonably explained by the subjects clinical state, environmental or toxic factors or other therapies administered to the subject
• followed a known pattern of response to the Investigational Product.

Unlikely: the adverse event
• did not follow a reasonable temporal sequence from administration of the Investigational Product
• could have been reasonably explained by the subjects clinical state, environmental or toxic factors or other therapies administered to the subject
• did not follow a known pattern of response to the Investigational Product
• did not reappear or worsen upon rechallenge.
Not classifiable:
- the adverse event’s causal relationship to use of Investigational Product could not have been judged because information was insufficient or contradictory, and could not be supplemented or verified.

The OUTCOME of the event was described in terms of the date of outcome, or the event was recorded as “ongoing”.

All adverse events persisting at the end of study and deemed clinically relevant were followed until it was deemed no longer relevant to do so.

9.15.3 Reporting of Serious Adverse Events

Any serious adverse event, related or unrelated to the Investigational Product occurring during the course of the study was reported to Leo Pharmaceutical Products within ONE working day after first knowledge by the investigator.

Reports were made using the Leo SERIOUS ADVERSE EVENT REPORT FORM, supplied by Leo. The information provided on the form included a description of the clinical course of the serious adverse event and an assessment of the intensity, relationship to the Investigational Product(s), the action taken and the outcome to date.

The initial report was followed by a detailed description later which may have included copies of hospital records, autopsy reports and other documents when requested and applicable.

If an investigator was in doubt whether to regard an adverse event as serious or not, the event was considered as serious until the opposite had been established.
The Independent Ethics Committee and National Health Authorities were to be notified on such an event in writing according to local law. All serious adverse event descriptions were also recorded on the adverse event page of the CRF book in addition to being reported on the Leo Serious Adverse Event Form.

9.16 QUALITY ASSURANCE

During all phases of the study, Leo Pharmaceutical Products implemented a system of quality assurance, including all the elements described in the protocol. Within this system, company Standard Operating Procedures (SOPs) were implemented to ensure that this clinical study was conducted in compliance with regulatory requirements and Good Clinical Practice. Quality control was applied to each stage of data handling to ensure that data were accurate, reliable and processed correctly.

Investigational sites, facilities, laboratories and all data (including sources) and documentation had to have been available for GCP audit by Leo Pharmaceutical Products or inspection by competent authorities. Any aspect of the trial was subject to audit by Leo Pharmaceutical Products and/or inspection by Regulatory Authorities (national or foreign) or IEC/IRB. Such audits/inspections may have taken place at the Sponsor’s site(s) or at any investigator’s site including laboratories, pharmacies etc.

The monitor, in case of audit, announced this in advance to the (sub)investigator and was present at the particular site during the audit. The site staff were expected to assist in all aspects of audit/inspection.
9.17 STATISTICAL ANALYSIS PLAN

Details of the statistical analysis planned prior to the start of the study are given in the Protocol, section 24.

At the end of the trial, prior to the treatment code being broken, a review of the proposed analysis was made and the relevant study populations were derived. Details of this are provided in the Statistical Analysis Plan Appendix II.

In accordance with the protocol, section 5, one subject who withdrew early from the study (CRF - ) was replaced with another subject (CRF ). This was done in order to maintain balance with respect to the randomization i.e. the 4 x 4 latin square design was maintained. All of the 16 subjects formed the randomised, intention-to-treat (ITT), per-protocol and safety populations. This decision was made after unblinding the study. The report therefore describes the data for 16 subjects. The data for the withdrawn subject is presented in the subject data listings, Appendix III.

A description of the results of this study is given in the main text of this report and statistical detail is given in the Statistical report (Appendix I). A summary of the statistical analyses planned in the protocol and the ones actually used, including when in the trial process decisions were made, is provided below.

Baseline comparability

No tabulations of baseline data were described in the protocol. At the time of the blind review it was decided to tabulate the baseline characteristics of the randomised subjects.
Efficacy analysis

All efficacy analyses were performed on the ITT population. The per-protocol population was identical to the ITT population (see Section 10.3 and Section 10.4).

The primary efficacy criterion was the change in skin thickness as measured by ultrasound in the areas of abdominal skin treated with each of the four treatments from baseline to each subsequent visit. The protocol stated that the change in skin thickness would be analysed by analysis of variance with repeated measures, with factors subject, treatment and time. At the blind review it was decided to also include treatment site as a factor in the model. The effect of time and the interaction of treatment and time was also assessed as described in the Statistical Analysis Plan.

The protocol stated that the change in skin thickness from baseline to end of treatment would be analysed by analysis of variance with factors subject and treatment. At the blind review it was decided to also include treatment site as a factor in the model.

Unaided visual assessment

The protocol stated that the unaided visual assessment (ordinal 5-point scale) would be analyzed by a non-parametric analysis of variance at each visit and end of treatment. At the blind review it was noted that the assessment was recorded as 0 ('no difference with surrounding skin') at each site, for all visits and for all subjects with the exception of one subject (CRF). Hence, it was decided no tables or analyses of these data were necessary.

Magnified visual assessment

The protocol stated that the magnified visual assessment (ordinal 5-point scale) would be analyzed by a non-parametric analysis of variance
at each visit and end of treatment. At the blind review it was noted that
the assessment was recorded as '0' ('no difference with surrounding
untreated skin') at each site, for all visits and for all subjects with the
exception of one subject (CRF [ ]). Hence, it was decided no tables or
analyses of these data were necessary.

Safety analysis
All safety analyses were performed on the safety population (see Section
10.5).

According to the protocol, adverse events were to be coded using the
WHO Adverse Reaction Dictionary. Adverse events were actually
coded according to the MedDRA (version 1.5) System Organ Class
(SOC) coding system and also by MedDRA preferred term. This
decision was made prior to unblinding the study.

For adverse events which affected treatment sites, the number of events
coded according to SOC and number and percentage of subjects with
adverse events was tabulated by treatment. If the same adverse event
was recorded on the same treatment site of the same subject on more
than one occasion, this was counted as one event for that treatment. A
similar table of adverse events reported by the MedDRA preferred term
by treatment was also produced.

At the blind review it was decided to also tabulate the intensity of
adverse reactions (i.e. adverse events where a causal relationship to
study treatment has not been excluded). Where the same adverse
reaction on the same site was reported at more than one visit by the
same subject, the "worst/highest" intensity reported was used in the
tabulations.
For adverse events which did not affect treatment sites, the number of events and number and percentage of subjects with adverse events according to SOC was tabulated.

9.18 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Changes to the planned analyses in the Protocol, made before the study was unblinded, are given in the Statistical Analysis Plan. There were no subsequent changes to any of the analyses described in the Statistical Analysis Plan.

There was one change to the populations described in the Statistical Analysis Plan. The last subject to enter the study (CRF) did so to replace a subject who withdrew (CRF), in accordance with the protocol section 5. The withdrawn subject was removed from all study populations. This decision was made after the study was unblinded.
10 RESULTS

10.1 STUDY PERIOD

10.1.1 Study Start
The first subject was recruited on Sept 26, 1996.

10.1.2 Study Completion
The last subject was recruited on November 8, 1996, and the last subject left the study on December 19, 1996.

10.1.3 Study Duration/Subject Recruitment
The study was completed over a total period of 3 months.

Individual subject data regarding time of recruitment, randomisation and withdrawal visit dates and time intervals between visits is presented in Appendix III, Table 1.
10.2 STUDY POPULATION

10.2.1 Disposition of Study Subjects

Fig.1: Flow Chart of Study Subject Disposition

10.2.1.1 Enrolment and randomisation of subjects
A total of 17 subjects were enrolled into the study. Subjects were enrolled by 3 investigators at 1 centre.

Of these subjects, all 17 were randomised at visit 1, all 17 received and used each of the 4 study medications, and all 17 were assessed at least at one further visit.

10.2.1.2 Withdrawal of subjects prior to randomisation
No subjects who were assessed at visit 1, were withdrawn from or left the trial before receiving any treatment.
10.2.1.3 Withdrawal of subjects from double-blind treatment

One of the 17 subjects randomised in the study, (CRF [Redacted]) was withdrawn from double-blind treatment. This subject was replaced (see CRF [Redacted]).

For individual data, see Appendix III, Table 13.

10.3 INTENTION-TO-TREAT POPULATION (ITT POPULATION)

Enrolled subjects were to be excluded from the ITT sample for efficacy analysis for any of the following reasons:

1. The subject was withdrawn from the trial prior to Visit 4, and therefore was replaced.
2. The subject was known to have taken the wrong trial medication throughout treatment.
3. The subject applied no trial treatment (this was absolutely confirmed).

NOTE: Subjects deviating from the protocol or violating eligibility criteria were included in the ITT population.

For this study, there were 17 enrolled subjects, and 16 subjects receiving each trial medication in the ITT population.

10.4 PER-PROTOCOL POPULATION (PP POPULATION)

The basis of the per-protocol population is the ITT population, from which further exclusions were made, including:

1. Subjects who did not fulfil all of the study protocol's inclusion criteria or who met any of the exclusion criteria.
2. Subjects who violated any other protocol criteria (for example compliance).
3. Other cases, when the situation was discussed and a course of action agreed upon prior to breaking the randomisation code.

For this study, there were 17 enrolled subjects, and 16 subjects receiving each trial medication in the per-protocol population.

10.5 SAFETY POPULATION

The basis of the safety population is the randomised population, from which further exclusions were made, including:
1. Subjects who were withdrawn and replaced.
2. Subjects who were lost to follow-up after visit 1. If such subjects were included, they would serve to artificially/incorrectly lower the apparent frequency of AEs. AE frequency is expressed on the basis only of those subjects for whom the AE data is known.
3. Subjects who had taken no trial medication (this was absolutely confirmed).

NOTE: Any subject who received wrong trial medication was accounted for with respect to the medication actually taken. Such cases are described in text and excluded from the main AE tables.

For this study, there were 17 enrolled subjects, and 16 subjects in the safety population for each treatment.
11  PROTOCOL DEVIATIONS

There were no identified/reported protocol deviations.

12  DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

12.1  BASELINE CHARACTERISTICS

The four tables below (Tables 1 - 4) present the baseline demographic characteristics of the study subjects. Each subject received all investigational products used in the trial, and thus acted as their own control in the analysis of the study.

Table 1  Age of Subjects

<table>
<thead>
<tr>
<th>AGE (years)</th>
<th>RANDOMISED POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>27.2</td>
</tr>
<tr>
<td>SD</td>
<td>7.6</td>
</tr>
<tr>
<td>Minimum</td>
<td>22</td>
</tr>
<tr>
<td>Maximum</td>
<td>54</td>
</tr>
<tr>
<td>Number</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 2  Sex of Subjects

<table>
<thead>
<tr>
<th>SEX</th>
<th>NUMBER OF SUBJECTS</th>
<th>% OF SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6</td>
<td>37.5</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>62.5</td>
</tr>
</tbody>
</table>
Table 3  Race of Subjects

<table>
<thead>
<tr>
<th>RACE</th>
<th>NUMBER OF SUBJECTS</th>
<th>% OF SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>15</td>
<td>93.8</td>
</tr>
<tr>
<td>African American /</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Black</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Oriental/Asian</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4  Skin Type of Subjects

<table>
<thead>
<tr>
<th>SKIN TYPE</th>
<th>NUMBER OF SUBJECTS</th>
<th>% OF SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
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<td>6.3</td>
</tr>
<tr>
<td>Type II</td>
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<td>18.8</td>
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<tr>
<td>Type III</td>
<td>8</td>
<td>50.0</td>
</tr>
<tr>
<td>Type IV</td>
<td>4</td>
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<tr>
<td>Type V</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Type VI</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

For individual data, see Appendix III, Table 2

12.2 VARIABILITY OF BASELINE CHARACTERISTICS OF STUDY SITES

This study involved only 1 centre, so there was no inter-site variability.

12.3 SKIN THICKNESS AT BASELINE

Skin thickness was assessed at baseline and all subsequent study visits. The randomisation process resulted in individual subjects applying the same medication to the same relative area of skin during the study, as set out in the template, but different subjects applying medication to different locations of the template. Thus, between subjects, each drug could be variously applied to Site A, B, C or D.
The baseline scores for thickness are presented in Table 5 below.

### Table 5 Baseline Skin Thickness (ultrasound measurement)

<table>
<thead>
<tr>
<th>SKIN THICKNESS (micron)</th>
<th>BETA-METHASONE (n=16)</th>
<th>CALCIPOTRIOL (n=16)</th>
<th>MOMETASONE (n=16)</th>
<th>VEHICLE (n=16)</th>
<th>UNTREATED SITE (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1620.3</td>
<td>1497.5</td>
<td>1596.8</td>
<td>1721.8</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>279.1</td>
<td>73.2</td>
<td>79.4</td>
<td>167.1</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>1346</td>
<td>1333</td>
<td>1497</td>
<td>1514</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1920</td>
<td>1471</td>
<td>1567</td>
<td>1891</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Site B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1715</td>
<td>1822.5</td>
<td>1790.3</td>
<td>1841.5</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>166.7</td>
<td>158.6</td>
<td>57.9</td>
<td>260.6</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>1562</td>
<td>1731</td>
<td>1716</td>
<td>1655</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1920</td>
<td>2013</td>
<td>1856</td>
<td>2155</td>
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</tr>
<tr>
<td>Number</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Site C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1879.5</td>
<td>1741.0</td>
<td>1862.5</td>
<td>1744.0</td>
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</tr>
<tr>
<td>SD</td>
<td>137.8</td>
<td>143.4</td>
<td>208.8</td>
<td>151.3</td>
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<tr>
<td>Maximum</td>
<td>1674</td>
<td>1623</td>
<td>1556</td>
<td>1554</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1961</td>
<td>1947</td>
<td>2007</td>
<td>1924</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Site D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1641.8</td>
<td>1741.0</td>
<td>1694.8</td>
<td>1592.0</td>
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</tr>
<tr>
<td>SD</td>
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<td>163.8</td>
<td>314.4</td>
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</tr>
<tr>
<td>Maximum</td>
<td>1464</td>
<td>1398</td>
<td>1287</td>
<td>1376</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1943</td>
<td>1920</td>
<td>2092</td>
<td>1760</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>All sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1714.1</td>
<td>1693.0</td>
<td>1736.0</td>
<td>1748.8</td>
<td>1654.9</td>
</tr>
<tr>
<td>SD</td>
<td>207.5</td>
<td>216.6</td>
<td>202.7</td>
<td>199.1</td>
<td>180.3</td>
</tr>
<tr>
<td>Maximum</td>
<td>1346</td>
<td>1305</td>
<td>1287</td>
<td>1376</td>
<td>1356</td>
</tr>
<tr>
<td>Minimum</td>
<td>1961</td>
<td>2013</td>
<td>2092</td>
<td>2195</td>
<td>1943</td>
</tr>
<tr>
<td>Number</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

For individual data, see Appendix III, Table 8.

### 13 EFFICACY RESULTS

#### 13.1 CHANGE IN SKIN THICKNESS SCORES - ULTRASOUND

The changes in skin thickness from baseline to each subsequent visit, are shown below in Table 6.
Table 6  Change in Skin Thickness (ultrasound measurement)

<table>
<thead>
<tr>
<th>SKIN THICKNESS (microns)</th>
<th>BETA-METHASONE (n = 16)</th>
<th>CALCIPOTRIOL (n = 16)</th>
<th>MOMETASONE (n = 16)</th>
<th>VEHICLE (n = 16)</th>
<th>UNTREATED SITES (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1714.1</td>
<td>1693.0</td>
<td>1736.0</td>
<td>1714.8</td>
<td>1654.8</td>
</tr>
<tr>
<td>SD</td>
<td>207.5</td>
<td>216.5</td>
<td>202.7</td>
<td>199.1</td>
<td>186.3</td>
</tr>
<tr>
<td>Minimum</td>
<td>1246</td>
<td>1303</td>
<td>1267</td>
<td>1375</td>
<td>1336</td>
</tr>
<tr>
<td>Maximum</td>
<td>1961</td>
<td>2015</td>
<td>2052</td>
<td>2195</td>
<td>1943</td>
</tr>
<tr>
<td>Number</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Change to</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-53.6</td>
<td>-3.9</td>
<td>-92.9</td>
<td>-25.4</td>
<td>-74.2</td>
</tr>
<tr>
<td>SD</td>
<td>109.9</td>
<td>124.4</td>
<td>117.7</td>
<td>124.7</td>
<td>100.4</td>
</tr>
<tr>
<td>Minimum</td>
<td>-244</td>
<td>-187</td>
<td>-245</td>
<td>-203</td>
<td>-310</td>
</tr>
<tr>
<td>Maximum</td>
<td>145</td>
<td>254</td>
<td>193</td>
<td>146</td>
<td>140</td>
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<tr>
<td>Number</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Change to</td>
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<td></td>
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<td>Visit 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-39.8</td>
<td>-10.6</td>
<td>-106.7</td>
<td>-39.0</td>
<td>-72.1</td>
</tr>
<tr>
<td>SD</td>
<td>141.5</td>
<td>124.4</td>
<td>109.3</td>
<td>96.6</td>
<td>107.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>-269</td>
<td>-173</td>
<td>-285</td>
<td>-170</td>
<td>-253</td>
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<td>Maximum</td>
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<td>201</td>
<td>154</td>
<td>185</td>
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<td></td>
</tr>
<tr>
<td>Visit 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>-111.1</td>
<td>-38.5</td>
<td>-130.7</td>
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<td>139.0</td>
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<td>-382</td>
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<td>-387</td>
</tr>
<tr>
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<td>Number</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>
This mean skin thickness irrespective of treatment area, at each visit (weeks 0, 2, 4 and 6) is shown graphically in Fig 2 below.

Figure 2. Mean Skin Thickness (Ultrasound)

For individual data, see Appendix III, Table 8.

The primary response criterion was the change in skin thickness, as measured by ultrasound, from baseline to each subsequent visit.

For the treated sites the mean change in skin thickness from baseline to subsequent visits is not the same for all treatments ($P=0.017$). The mometasone treated sites had a far larger degree of skin thinning compared to the other treatment sites. The mean change in skin thickness from baseline to each subsequent visit is similar for sites treated with betamethasone, calcipotriol and vehicle of calcipotriol. There was no statistically significant effect of time ($P=0.85$) and no evidence of any treatment by time interaction ($P=0.91$). The mean skin
thickness for the untreated site decreased with each subsequent visit. The mean change in skin thickness from baseline to visit 4 was greatest for the untreated site.

The mean differences and 95% confidence intervals between each pair of treatments for the change in skin thickness from baseline to end of treatment are presented in Table 7.

**Table 7** Pairwise comparisons of change in skin thickness from baseline to end of treatment.

<table>
<thead>
<tr>
<th>TREATMENT COMPARISON</th>
<th>MEAN END-OF-TREATMENT DIFFERENCE (MICRONS) (N=16)</th>
<th>95% CONFIDENCE INTERVAL</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcipotriol - Betamethasone</td>
<td>41.1</td>
<td>(-36.3 to 118.4)</td>
<td>0.29</td>
</tr>
<tr>
<td>Calcipotriol - Mometasone</td>
<td>123.1</td>
<td>(45.8 to 200.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>Calcipotriol - Vehicle</td>
<td>50.5</td>
<td>(-26.8 to 127.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>Betamethasone - Mometasone</td>
<td>82.1</td>
<td>(-4.7 to 159.4)</td>
<td>0.038</td>
</tr>
<tr>
<td>Vehicle - Mometasone</td>
<td>72.6</td>
<td>(-4.7 to 150.0)</td>
<td>0.065</td>
</tr>
<tr>
<td>Vehicle - Betamethasone</td>
<td>-9.4</td>
<td>(-86.8 to 67.9)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The largest mean difference at end-of-treatment treatment was 123.1µ (95% CI 45.8 to 200.5) for the comparison of calcipotriol with mometasone.

### 13.2 CHANGE IN SKIN THICKNESS SCORES - UNAIDED VISUAL ASSESSMENT

For all subjects, the response for UNAIDED VISUAL ASSESSMENT was 'No difference compared with surrounding skin' at each visit for each area (A-D) with the following exception:
CRF area A (mometasone treated area) at visit 3 and visit 4 had "Slight thinning, slight smoothing of the surface relief and only just visible increase in transparency."

For individual data, see Appendix III, Table 6.

13.3 CHANGE IN SKIN THICKNESS SCORES - MAGNIFIED VISUAL ASSESSMENT

For each subject the response for MAGNIFIED VISUAL ASSESSMENT was 'No difference compared with surrounding untreated skin' at each visit for each area (A-D) with the following exception:

CRF area A (mometasone treated area) at visit 3 and visit 4 had "Capillary hyperaemia with weak elongation and dilatation of a few vessels. Not visible with the naked eye."

For individual data, see Appendix III, Table 7.

14 SAFETY EVALUATION

14.1 ADVERSE EVENTS REPORTED

The design of the study was such that each subject received each treatment - i.e., except for the exact location of the treatments, each subject was treated the same. As a result, unless an Adverse Event was only associated with 1 or more specific treatment locations within the template, it was reported as being associated with none of the treatments for a given subject.

In an effort to present the data in as meaningful a manner as possible, AEs are therefore divided into two groups:
• those associated with 1 or more specific treatment area(s), and
• those not so associated.

The treatment area affected by a particular adverse event was recorded on the CRF. These AEs have been tabulated by treatment group.

The treatment area associated AEs relating to a particular treatment group have been classified according to their MedDRA SOC (system organ class) group, presented in Table 8, and by the MedDRA preferred term, presented in Table 9.

It should be noted that in some cases, there were AEs that were "treatment area associated" but were judged not to be related to use of the treatment (i.e., they were not ADRs).

Table 8 Treatment area associated Adverse Events - MedDRA SOC group

<table>
<thead>
<tr>
<th></th>
<th>BETA-METHASONE (n = 15)</th>
<th>CALCIPOTRIOL (n = 16)</th>
<th>MOMETASONE (n = 16)</th>
<th>VEHICLE (n = 16)</th>
<th>TOTAL AEs (n = 16)</th>
<th>TOTAL SUBJECTS (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections &amp; infestations</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Skin &amp; Subcutaneous tissue disorders</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total number of adverse events</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total number of subjects (%)</td>
<td>1 (6.3)</td>
<td>0 (0.0)</td>
<td>1 (6.3)</td>
<td>1 (6.3)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
The same AEs have been classified according to their MedDRA preferred term, shown in Table 9.

Table 9  Treatment area associated Adverse Events - MedDRA Preferred term

<table>
<thead>
<tr>
<th></th>
<th>BETA-METHASONE (n=16)</th>
<th>CALCIPOTRIOL (n=16)</th>
<th>MOMETASONE (n=16)</th>
<th>VEHICLE (n=16)</th>
<th>TOTAL AEs</th>
<th>TOTAL SUBJECTS (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folliculitis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rash erythematosus</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total number of adverse events</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total number of subjects (%)</td>
<td>1 (6.3)</td>
<td>0 (0.0)</td>
<td>1 (6.3)</td>
<td>1 (6.3)</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

The degree of suspected relationship of the treatment to the reported adverse events, listed by MedDRA preferred terms is shown in Tables 10a and 10b.

Table 10a  Degree of suspected causality of treatment area associated Adverse Events (AEs)

<table>
<thead>
<tr>
<th></th>
<th>BETAMETHASONE (n=16)</th>
<th>CALCIPOTRIOL (n=16)</th>
<th>Unlikely</th>
<th>Possible</th>
<th>Probable</th>
<th>Unlikely</th>
<th>Possible</th>
<th>Probable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folliculitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash erythematosus</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total AEs</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 10b  Degree of suspected causality of treatment area associated Adverse Events (AEs)

<table>
<thead>
<tr>
<th></th>
<th>MOMETASONE (n=16)</th>
<th>VEHICLE (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlikely</td>
<td>Possible</td>
</tr>
<tr>
<td>Folliculitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash erythematous</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The severity of the ADR's occurring in the area treated with study medication are listed by MedDRA preferred terms and shown in Tables 11a and 11b.

Table 11a  Severity of Treatment area associated Adverse Reactions (ADRs)

<table>
<thead>
<tr>
<th></th>
<th>BETAMETHASONE (n=16)</th>
<th>CALCIPOTRIOL (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td>Folliculitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash erythematous</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total ADRs</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 11b  Severity of Treatment area associated Adverse Reactions

<table>
<thead>
<tr>
<th></th>
<th>MOMETASONE (n = 16)</th>
<th>VEHICLE (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td>Number of adverse reactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folliculitis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rash erythematous</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total ADRs</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

The AEs not associated with a particular treatment area are shown in Table 12.

Table 12  Adverse events not associated with/limited to treatment areas

<table>
<thead>
<tr>
<th>Adverse Events (MedDRA SOC)</th>
<th>Number of subjects (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General disorders</td>
<td>1</td>
</tr>
<tr>
<td>Infections &amp; infestations</td>
<td>3</td>
</tr>
<tr>
<td>Injury &amp; poisoning</td>
<td>2</td>
</tr>
<tr>
<td>Musculoskeletal connective tissue &amp; bone disorder</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory thoracic &amp; mediastinal disorders</td>
<td>1</td>
</tr>
<tr>
<td>Skin &amp; subcutaneous tissue disorders</td>
<td>1</td>
</tr>
<tr>
<td>Surgical &amp; medical procedures</td>
<td>1</td>
</tr>
<tr>
<td>Total number of subjects with adverse events (%)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Total number of adverse events</td>
<td>10</td>
</tr>
</tbody>
</table>

There is no evidence of any difference between treatments with respect to any type of adverse event.

For individual data, see Appendix III, Table 11 and 12.
14.2 DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

A single subject, CRF [REDACTED] was removed from the trial due to an emergency appendectomy being performed. This was judged to have no causal relationship with the study drug. The subject was replaced with CRF [REDACTED].

14.3 LABORATORY EXAMINATION

No safety laboratory examinations were made, beyond the assessment of skin atrophy which is presented in the efficacy section.
15 USE OF INVESTIGATIONAL PRODUCT

15.1 USE OF AND COMPLIANCE WITH INVESTIGATIONAL PRODUCT

At each visit, subjects were asked to return the medication dispensed at the prior visit, and tubes were subsequently weighed to determine amounts used. No information from subjects was collected regarding daily use of the medication.

The weight of each investigational product used during the trial is presented in Table 13.
Table 13  Amount of study medication (grams) used between visits during the study

<table>
<thead>
<tr>
<th></th>
<th>Betamethasone (n=16)</th>
<th>Calcipotriol (n=16)</th>
<th>Mometasone (n=16)</th>
<th>Vehicle (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites VISIT 1 TO VISIT 2</td>
<td>Mean 2.4 2.9 2.5 3.7</td>
<td>Mean 1.9 2.7 1.9 2.1</td>
<td>Mean 1 0 0 2</td>
<td>Mean 8 7 8 8</td>
</tr>
<tr>
<td></td>
<td>SD 2.5 1.9 2.1 1.9</td>
<td>Maximum 10 8 7 8</td>
<td>Maximum 16 16 16 16</td>
<td>Maximum 16 16 16 16</td>
</tr>
<tr>
<td></td>
<td>Minimum 0 1 0 2</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
</tr>
<tr>
<td>VISIT 2 TO VISIT 3</td>
<td>Mean 1.5 2.2 2.0 3.0</td>
<td>Mean 1.6 1.6 1.5 1.2</td>
<td>Mean 0 0 0 2</td>
<td>Mean 5 5 5 6</td>
</tr>
<tr>
<td></td>
<td>SD 1.6 1.6 1.5 1.2</td>
<td>Maximum 4 5 5 6</td>
<td>Maximum 16 16 16 16</td>
<td>Maximum 16 16 16 16</td>
</tr>
<tr>
<td></td>
<td>Minimum 0 0 0 2</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
</tr>
<tr>
<td>VISIT 3 TO VISIT 4</td>
<td>Mean 2.3 3.1 3.1 3.9</td>
<td>Mean 2.5 3.5 3.5 3.5</td>
<td>Mean 0 0 0 2</td>
<td>Mean 7 7 7 7</td>
</tr>
<tr>
<td></td>
<td>SD 0.3 0.3 0.3 0.3</td>
<td>Maximum 11 10 10 10</td>
<td>Maximum 16 16 16 16</td>
<td>Maximum 16 16 16 16</td>
</tr>
<tr>
<td></td>
<td>Minimum 0 0 0 0</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
</tr>
<tr>
<td>VISIT 4 TO EOT</td>
<td>Mean 7.1 8.1 7.6 10.7</td>
<td>Mean 5.5 5.6 5.2 4.7</td>
<td>Mean 0 0 0 6</td>
<td>Mean 3 1 1 6</td>
</tr>
<tr>
<td></td>
<td>SD 0.3 0.3 0.3 0.3</td>
<td>Maximum 9 9 9 9</td>
<td>Maximum 25 21 21 21</td>
<td>Maximum 16 16 16 16</td>
</tr>
<tr>
<td></td>
<td>Minimum 0 0 0 0</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
<td>Number 16 16 16 16</td>
</tr>
</tbody>
</table>

For individual data, see Appendix III, Table 16.

15.2 DURATION/EXTENT OF EXPOSURE TO INVESTIGATION PRODUCT

Each subject applied all investigational products, therefore the duration of treatment with each drug was the same (6 weeks).
CONCOMITANT TREATMENT

Each subject applied all investigational products, therefore the concomitant use of non-investigational products with investigational products was the same. Table 14 below shows the use of concomitant treatment during the study.

Table 14  Concomitant Treatment

<table>
<thead>
<tr>
<th>ANATOMICAL THERAPEUTIC CHEMICAL (ATC) CLASSIFICATION INDEX</th>
<th>ALL RANDOMISED SUBJECTS</th>
<th>(n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of drugs</td>
<td>Number of subjects</td>
</tr>
<tr>
<td>Alimentary tract and metabolism</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>General anti-infectives — systemic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Genito-urinary system and sex hormones</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Musculo-skeletal system</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Various</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total number of drugs taken</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Total number (% ) of subjects taking drugs</td>
<td></td>
<td>14 (87.5)</td>
</tr>
</tbody>
</table>

For individual data, see Appendix III, Table 3.
17 DISCUSSION

The objective of the study was to compare the safety, in terms of the degree of skin atrophy induced, of twice daily calcipotriol ointment (50 ug/g), twice daily betamethasone 17-valerate ointment (1 mg/g), once daily mometasone furoate ointment (1 mg/g) and twice daily use of the vehicle of calcipotriol ointment. All products (except the vehicle of calcipotriol ointment) are commonly used in the treatment of chronic plaque psoriasis.

The mean change in skin thickness from baseline to subsequent visits was not the same for all treatments ($P=0.017$). The mean change in skin thickness induced by mometasone was greater than the mean change induced by the other treatments at each visit.

The change in skin thickness from baseline to end of treatment induced by mometasone was shown to be significantly greater than the change induced by both betamethasone and calcipotriol ($P=0.038$ and $P=0.003$ respectively). Conversely, the study was unable to demonstrate a statistically significant difference between the atrophogenic properties of calcipotriol, betamethasone 17-valerate and the vehicle of calcipotriol ointments.

17.1 STUDY DESIGN, APPROVAL AND CONDUCT

The study was a single-centre, prospective, randomised, double blind, within-subject study. A total of 17 subjects were randomised to receive all study drugs. One subject was withdrawn from the study due to the need for non-study related surgery (appendectomy). In accordance with the protocol, this subject was replaced with another subject who was assigned a treatment pattern in the same order as the original subject. (The data from the replaced subject was not included in any of
the tables in the report. It is included in the various line listing of data of all subjects data in Appendix III).

Prestudy sample size calculations were based on an assumption that the intra subject standard deviation would maximally be 0.25 mm and that an observed difference of 0.25 mm represented a significant change in skin thickness. Based on these original assumptions, the sample size calculations showed a requirement for a total of 16 evaluable subjects, with each subject receiving all investigational products.

The actual observed SD was 0.087mm in the calcipotriol arm, 0.091mm in the betamethasone arm, 0.095mm in the mometasone arm and 0.071mm in the vehicle arm. This was less than the original expectations. The standard deviations calculated in the tables showing changes in skin thickness are quite large compared to the actual size of the mean changes observed. This is an expected finding, routinely associated with parameters measuring a "change" induced by a given treatment/intervention.

The statistical plan incorporated an assumption of normal distribution of subject data. This assumption was not specifically tested beyond a visual inspection of the data, which appeared normally distributed.

The investigational product was similar in colour, feel, smell and texture. The labelling did not provide insight into the contents of either tube, and the randomisation envelopes were not opened, with the exception of the "replaced" subject. Thus, the trial was effectively fully blinded for the primary efficacy criterion.

The study protocol was reviewed and approved by the National Health Authorities of Canada. Additionally, the centre received written approval of the study protocol and subject related documents (informed
consent, subject information) from an Institutional Review Board prior to starting the trial. Overall responsibility for ensuring that the project was conducted in a matter that met the GCP requirements was placed with a medically qualified clinical project manager of Leo Pharmaceutical Products.

The study was conducted on a single-centre basis in Canada. Staff at the investigational site were provided with written information (protocol, CRF, subject documents), and provided detailed, study specific training by company personnel. Prior to study initiation, a final pre-study visit was made by company personnel to verify that all matters pertaining to the study were well understood by the centre, and that all required documentation was on site. The site was routinely monitored on a schedule related to subject enrolment, and written documentation was made of each monitoring visit. Following verification of the data base, the investigative centre was informed of their post-trial obligations regarding document storage, subject follow-up and IRB notifications.

All subjects were diagnosed, assessed and treated by qualified dermatologists. No subject was known nor judged to have received any therapy that would significantly affect the study results regarding the atrophogenic properties of the treatments.

Subjects applied the various drugs to a specific area defined by a template, and were provided supplies of gloves to avoid transfer of one study drug to an area it was not intended for. Subject compliance with respect to using the investigational product appeared adequate.

A single subject was replaced, according to the protocol instructions, due to the requirement for non-study related surgery. The subject who replaced the withdrawn subject received the same medication scheme, in terms of application area of each treatment, as the withdrawn subject.
The data from the withdrawn subject was not included in the analysis. Otherwise, no randomised subject violated any inclusion/exclusion, and all had their data included in the safety and efficacy analysis.

Therefore, the design, approval and conduct of the study can be declared to have met ICH GCP and regulatory requirements.

17.2 RESULTS

The primary response criterion in the study was:

The change in skin thickness, as measured by ultrasound, in the areas of abdominal skin treated with each of the four treatments, from baseline to each subsequent visit.

This repeated measures analysis incorporated the change from baseline to each subsequent visit in the determination of the existence and size of differences between the effects of the various treatments.

Table 6 shows a summary of mean changes in skin thickness to each visit. Across the entire study there was a statistically significant difference between treatments in the change in skin thickness (P=0.017). The greatest degree of skin thinning was with mometasone; the other two treatments (and vehicle) showed similar amounts of skin thinning.
A supplementary analysis was also performed, wherein the change in skin thickness from baseline to end-of-treatment was analysed between treatment groups. This analysis only incorporated the change from baseline to end-of-treatment measurements in the determination of the existence and size of differences between the various treatments.

There were no differences noted in the results of the above 2 analyses. The absence of any time by treatment effect supports other findings that the change (decrease) in skin thickness induced by corticosteroids occurs very quickly.

### Table 6  Change in Skin Thickness (abridged)

<table>
<thead>
<tr>
<th>SKIN THICKNESS (micron)</th>
<th>BETA-METHASONE (n=16)</th>
<th>CALCIFOTRIOL (n=16)</th>
<th>MOMETASONE (n=16)</th>
<th>VEHICLE (n=16)</th>
<th>UNTREATED SITE (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1714.1</td>
<td>1693.0</td>
<td>1736.0</td>
<td>1714.8</td>
<td>1654.9</td>
</tr>
<tr>
<td>SD</td>
<td>207.5</td>
<td>216.6</td>
<td>202.7</td>
<td>199.1</td>
<td>188.3</td>
</tr>
<tr>
<td>Change to Visit 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-53.6</td>
<td>-3.9</td>
<td>-93.9</td>
<td>-25.4</td>
<td>-76.2</td>
</tr>
<tr>
<td>SD</td>
<td>109.9</td>
<td>124.4</td>
<td>117.7</td>
<td>124.7</td>
<td>102.4</td>
</tr>
<tr>
<td>Change to Visit 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-39.8</td>
<td>-10.6</td>
<td>-106.7</td>
<td>-39.0</td>
<td>-72.1</td>
</tr>
<tr>
<td>SD</td>
<td>141.5</td>
<td>124.4</td>
<td>109.3</td>
<td>96.6</td>
<td>107.8</td>
</tr>
<tr>
<td>Change to Visit 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-29.1</td>
<td>12.0</td>
<td>-111.1</td>
<td>-34.5</td>
<td>-130.7</td>
</tr>
<tr>
<td>SD</td>
<td>129.0</td>
<td>139.0</td>
<td>124.2</td>
<td>106.6</td>
<td>111.0</td>
</tr>
</tbody>
</table>

As a supplementary analysis was also performed, wherein the change in skin thickness from baseline to end-of-treatment was analysed between treatment groups. This analysis only incorporated the change from baseline to end-of-treatment measurements in the determination of the existence and size of differences between the various treatments.
The secondary response criteria were the investigators’ assessment of atrophy by both:

- the unaided visual examination and
- the magnified visual examination.

Tables 6 and 7 in the Results section show the primary response criterion data, and show that, at the end of treatment, 3 of the 4 ointments applied resulted in skin thinning. The differences between the various treatments are shown in the table below.

Table 7  Change in skin thickness from baseline to end of treatment for all pairwise comparisons

<table>
<thead>
<tr>
<th>TREATMENT COMPARISON</th>
<th>MEAN END-OF-TREATMENT DIFFERENCE MICRONS (n = 16)</th>
<th>95% CONFIDENCE INTERVAL</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcipotriol - Betamethasone</td>
<td>41.1</td>
<td>(-36.3 to 118.4)</td>
<td>0.29</td>
</tr>
<tr>
<td>Calcipotriol - Mometasone</td>
<td>123.1</td>
<td>(45.8 to 200.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>Calcipotriol - Vehicle</td>
<td>50.5</td>
<td>(-26.8 to 127.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>Betamethasone - Mometasone</td>
<td>82.1</td>
<td>(-4.7 to 191.4)</td>
<td>0.038</td>
</tr>
<tr>
<td>Vehicle - Mometasone</td>
<td>72.6</td>
<td>(-4.7 to 150.0)</td>
<td>0.065</td>
</tr>
<tr>
<td>Vehicle - Betamethasone</td>
<td>-9.4</td>
<td>(-86.8 to 67.9)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Mometasone valerate was significantly different compared to both calcipotriol and betamethasone regarding the degree of skin thinning induced during treatment. In each case, the degree of atrophy induced by mometasone was greater than that induced with the comparator. Neither mometasone nor betamethasone valerate produced a statistically significant degree of atrophy compared with the vehicle.

There was no significant difference between the effects of the two tested corticosteroids and the vehicle regarding the ability to induce skin atrophy (P=0.065 and P=0.81 for mometasone and betamethasone respectively). This finding is not intuitive, in that a vehicle is generally
considered as a "placebo", and so would not be expected to cause any atrophy. During treatment, the vehicle treated area showed a mean decrease in thickness of 38.5 microns. The lack of significant difference between the corticosteroids and the vehicle is therefore not due to unexpectedly low effects of the steroids, but instead due to unexpected high effects of what was considered a "placebo" vehicle. Interestingly enough, at least 3 other investigations (12, 48, 49) have also independently found that application of a vehicle preparation resulted in a thinning of the skin. No explanations for the decrease were presented in the references.

Calcipotriol induced a small increase in mean skin thickness at visit 4, compared to baseline. Associated with this, there was a significant difference between the effects observed with mometasone and calcipotriol, and a trend towards a difference between the effects of the calcipotriol and both the vehicle and betamethasone. This finding matched those observed by other investigators using ultrasound measurements to investigate the skin thinning properties of various treatments (18). The other investigators attributed the post-calcipotriol application skin thickening to the onset of an irritant dermatitis. The possible etiology of the increase in thickness was not investigated in the current study, but the known properties of calcipotriol make the suggestion of a dermatitis seem a likely explanation.

The most difficult finding to reconcile in the study is that of the marked skin thinning observed in the untreated area. The area that was untreated and sequentially measured was defined by a template, so there was as much consistency in measurement in this area as any other (treated) area that was investigated. The same technician made all ultrasound assessments for all subjects on all occasions. However, we are unable to explain these results.
The untreated area lay below the 2 areas of treatment on the right hand side of the subject, at the level of the subject's navel. There may have been some effect on skin thickness through contamination of the untreated site by medication spread from above. Other, previous studies, in an effort to avoid possible contamination of other sites, required the removal of ointments from treated, occluded areas after 1 hour (12, 50). These studies used, as rational, data showing that following short term occlusion of treatment sites (1 hour), the stratum corneum reservoir was saturated with corticosteroid molecules. Such a design was not used in this trial, and therefore some contamination of the "untreated" areas may have inadvertently occurred. With 3 of the 4 treatments showing at least a trend to decrease skin thickness, contamination of the "untreated" area may have contributed to the observed decrease in skin thickness.
CONCLUSIONS

The application of corticosteroid ointments induced a thinning of the skin that was either significantly greater than that of calcipotriol (mometasone furoate applied once daily), or showed a trend to exceed the effect of calcipotriol (betamethasone valerate applied twice daily). However because of the difficulties in interpreting the data from the 'control (untreated) site' the results of the study cannot be considered reliable.
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