A Randomized, Double-blind Placebo-controlled Clinical Trial on the Efficacy and Safety of Adapalene 0.1% Gel on the Closure of Neuropathic Ulcer among Leprosy Patients

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INTRODUCTION

Leprosy is a chronic infectious disease principally affecting the skin, eyes, and the peripheral nerves. It is caused by the acid-fast, rod-shaped bacillus *Mycobacterium leprae*.[1] According to the World Health Organization, three million persons are suffering from the burden of the disease.[2] Damage to peripheral nerves is a key feature of leprosy and the motor and sensory loss that follow the disease is the basis of developing wounds, fissures, ulcerations, clawed hand, foot drop, and lagophthalmos. Neuropathic ulcers (NU) are one of the most common complications of leprosy seen in 10% of patients.[3] NU occur due to impairment of the sensory, motor, and autonomic nerve functions leading to lowered threshold for tissue damage.[4] They may arise in any part of the body and are divided into 2 groups: plantar and extra-plantar. Plantar ulcers, also called trophic ulcers or malum perforans pedis, are more common, comprising 70% of all NU. Ulcers occurring in other denervated sites, such as the dorsum and lateral malleolar region of the foot and hands are described under extra-plantar ulcers.[3] Aside from the loss of protective sensation on the foot, other factors such as repetitive moderate stress, callus formation, direct trauma, pressure, burns, and infection contribute to the development of NU.[5]

Despite recent and emerging advances in combating leprosy, management of NU in leprosy has taken a backseat. A recent systematic review of randomized controlled trials involving anyone with leprosy and damage to peripheral nerves treated with any measures designed to prevent damage with the aim of healing existing ulcers and preventing development of new ulcers identified eight trials with a total of 557 participants.[6] The quality of the trials was generally poor and interventions and outcome measures were diverse. Strategies in the approach to these frustrating ulcers have mainly been supportive including antibiotics, various wound dressings, and use of corrective shoes. Clinical trials exist in ulcer closure but these are few and deemed lacking.

The pathophysiology of chronic non-healing ulcers such as NU is marked by excessive infiltration of neutrophils that release significant amounts of enzymes such as collagenase or matrix metalloproteinase -8 (MMP-8) that is responsible for the breakdown of connective tissue matrix, and elastase that is capable of destroying essential healing factors such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-ß). Another marker of these chronic ulcers is an environment containing excessive reactive oxygen species that further damage the cells and healing tissues.[7]

The collagen molecule is characterized by repeating sequences of proline and hydroxyproline (HP). Collagen is important in all stages of wound healing and is critical in regaining tissue integrity and

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strength. In particular, hydroxyproline is important because it gives the collagen molecule its stable helical configuration.[7] The initial cleavage of collagen by MMP-8 represents the rate-limiting step in the degradation of this extracellular matrix protein. In non-healing ulcers, there is increased collagenolytic activity, reflected by elevated levels of MMP-8, decreased HP, and lower levels of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1). These findings suggest that overexpression and activation of MMP-8 may be involved in the pathogenesis of non-healing wounds.[8]

Retinoids have broad uses in dermatology and their role in wound healing has been widely studied. Preoperative use of retinoids is believed to enhance wound healing. However, there are mixed reviews regarding its efficacy in fresh and healing wounds. Vitamin A is an essential fat-soluble vitamin that plays an integral role in the maintenance of normal epidermis by promoting desquamation and maturation through a decrease in the production of keratin, keratohyalin granules, and desmosomes. Moreover, vitamin A also enhances would healing by stimulating angiogeneseis, collagen synthesis, and epithelialization.[9] Retinoids are synthetic and natural derivatives of vitamin A. They exert their activity on keratinocytes by binding to nuclear receptors, thereby regulating gene expression. .

Topical tretinoin (all-*trans*-retinoic acid) has been found to have positive effects on wound healing.[10] The use of tretinoin on a poorly granulating wound bed increases epidermal thickness and cell turnover, leading to faster reepithelialization and granulation tissue formation.[11] It also stimulates angiogenesis in the superficial dermis which allows efficient delivery of oxygen and nutrients to the tissues. Endothelial cells secrete biologically active substances that include growth factors.[10] Moreover, tretinoin also increases granulation tissue formation by increasing collagen synthesis by way of downregulating MMP-8.[12]

A randomized, double-blind, clinical trial by Tom et al. assessed the efficacy of short-contact tretinoin versus placebo.[10] The study showed that applying tretinoin 0.0.5% solution for 10 minutes on diabetic foot ulcers once a day for 4 weeks was superior to placebo saline solution. A similar technique was used in a clinical trial by Chua et al. where short-contact (10 minutes) tretinoin 0.05% cream improved the healing of foot ulcers in leprosy patients versus the placebo cream.[13] However, irritation caused by topical tretinoin is often a concern. Short-contact application of tretinoin may cause mild to moderate pain on the surrounding skin and ulcer itself.[10] Also, tretinoin is relatively more expensive compared to other wound care medications limiting its use.

Adapalene is a new naphthoic acid derivative with strong retinoid agonistic properties and belongs to the third-generation retinoids. It is also known to behave similarly to tretinoin.[14] Animal studies suggest that adapalene contributes to the wound healing process resulting in enhancement of collagen production, angiogenesis, and granulation tissue formation.[14-15] It is also noted that adapalene is more stable and is less irritating than tretinoin because of its unique receptor specificity.[15]

Ulceration of the feet is the single most common cause of disability and dehabilitation of patients with leprosy. Because of its impact in the patient's physical, emotional, and social health, it is of utmost importance that there be continuing search for a solution. A simple and well-tolerated treatment that affords closure of ulcer will prevent further ulceration, susceptibilty to infection, risk of amputation, and ultimately, disability. Currently, there are no existing studies about the use of topical adapalene on neuropathic ulcers in leprosy patients. A randomized controlled trial is therefore needed to determine the efficacy and safety of a retinoid derivative such as adapalene that is similar to tretinoin in pharmacologic action but is cheaper, more stable, and with less adverse effects.

OBJECTIVES

The general objective of the study is to determine the efficacy and safety of adapalene 0.1% gel versus placebo in the closure of neuropathic ulcer among leprosy patients.

The specific objectives are to determine if patients treated with adapalene 0.1% gel compared to

those given placebo gel were significantly different in terms of 1.) Percent change of ulcer surface area from baseline to post-treatment, and 2.) Number of weeks for the ulcer to close, and 3.) Incidence of adverse events.

MATERIALS AND METHODS

Patients and Study Design

This is a prospective, randomized, double-blind, placebo-controlled clinical trial on leprosy patients who present with neuropathic ulcer, plantar or extra-plantar, at the Dermatology Outpatient Department of Jose R. Reyes Memorial Medical Center.

Patients recruited included male or female patients both newly or previously diagnosed with leprosy who presented with neuropathic ulcer, plantar or extra-plantar, treated or currently under multidrug therapy (paucibacillary or multibacillary) with at least two weeks washout period from any oral antibiotics or topical medications before the study enrollment period.

Excluded from the study were patients unable to give informed consent, with known bleeding disorders, pregnant or breastfeeding at the time of enrollment, who have infected ulcers or nearby tissues at time of enrollment, who have other systemic disorders that will impede wound healing such as diabetes mellitus, with clinical and radiological signs of osteomyelitis, and who have a known or documented allergy to adapalene and other retinoid derivatives.

A certificate of approval from the Institutional Review Board of Jose R. Reyes Memorial Medical Center was obtained prior to starting the clinical trial. Written informed consent was secured before initiating treatment.

Materials

Both adapalene 0.1% gel and placebo gel were obtained from local companies. Both were repackaged and put in a plastic opaque jar and labeled by numbers. Placebo gel was specially made so that it would be identical to the treatment in terms of color, texture, and odor.

Randomization, treatment allocation, and blinding

Randomization was performed by an uninvolved third party who did a computer-generated random sequence to balance the participants of the 2 treatment groups. The study was double-blind in such a way that all dispensed jars were similar in appearance and that neither the patient nor the investigator was aware to which the patient was assigned until after the completion of the study.

Study Intervention

The patients were instructed to clean the wound bed with cotton swab moistened with normal saline solution before applying adapalene 0.1% gel or placebo gel. They would then apply the assigned gel directly on the wound and leave it on for 10 minutes with special instructions to avoid surrounding normal skin. After 10 minutes, patients would rinse it off with normal saline solution. This procedure was done once a day for 4 weeks. The participants were allowed to receive routine wound care such as protection of the ulcerated area with appropriate dressings. Patients were also advised to wear open-toed soft sandals as standard protective footwear. Accepted footwear was defined as having a soft insole about 1 cm thick, a hard sole that cannot be pierced by sharp objects such as stones and thorns, and an upper that fits well and has straps or laces over the forefoot that can be loosened. These were provided by the investigator to preserve uniformity.

Clinical Assessment

Photographs of the ulcers were taken to evaluate and document initial size and appearance. The photographs were taken using Nikon Coolpix P310 under standardized lighting and positioning of the patient. Photographs and measurement of ulcer size and volume were taken every 2 weeks for a maximum of 16 weeks after the start of the study or until complete healing occurs, whichever occurred first. Manual planimetry, wherein the ulcer is traced on acetate with grids, was used to measure surface area. The number of grids that fit within the traced circumference was counted and multiplied by area in square centimeters to yield the surface area.[18] All partial grids divided by the tracing line by more than half were likewise included.

Primary endpoint is determined as 50% or greater reduction in the percent change in ulcer surface area and volume and from baseline until the end of the study. Secondary endpoint is defined as the number of weeks at which reduction in ulcer surface and volume was observed. Both endpoints will be assessed at every visit.

Any untoward effects encountered during the treatment such as erythema, edema, purulence, and burning sensation were noted.

Stopping guidelines

The study was to be stopped in patients who experienced excessive irritation defined as burning sensation, diffuse erythema, and pain on application. These patients were considered as withdrawals from the study. Those who did not comply with the treatment regimen, or those who used other topical medications were withdrawn from the study.

Drop-outs were defined as those who did not follow up within two weeks and whose outcome was unknown by the end of the study period.

Sample size

Since there is a small population of leprosy patients with neuropathic ulcer at Jose R. Reyes Memorial Medical Center, sample size was inferred from a similar clinical trial done at our institution which had 16 participants. For this study, sample size was doubled to 32 patients, with 16 participants in each study arm.

Data Processing and Analysis

Primary endpoint was computed as the percent change of the ulcer surface area from baseline to the end of the study. The differences between the size of the ulcer from the baseline to week 16 were assessed using analysis of variance (ANOVA) of repeated measures. A Post-Hoc Test, Tukey test, was used when ANOVA showed a significant result. Statistical analysis was performed with the use of STATA software (version 12.0). A p value < 0.05 was considered statistically significant.

RESULTS

Study Population

Thirty-eight patients were evaluated for eligibility between June 2013 and May 2014. Two did not qualify for the study because of the presence of infected ulcers; another 2 patients diagnosed with diabetes mellitus were excluded; and 2 patients who did not meet any exclusion criteria refused to participate in the study. A total of 32 patients were enrolled in the study, with 16 patients assigned to each arm **(Figure 1)**. There were no withdrawals nor drop-outs during the study.

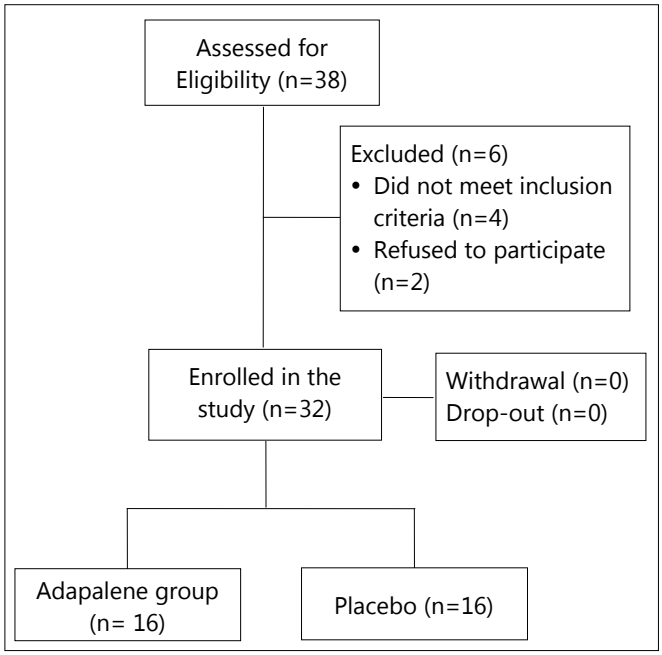

Figure 1. Patient Disposition

Majority of the subjects were comprised of males (75%) with 81.25% (13/16) from the treatment group and 68.75% (11/16) from the control group. Mean age for both groups was 55.18, 55.43 years for treatment group while 54.93 years for the control group. Mean size of the ulcer on baseline was 8.25 cms, measuring 9.875 cms for the treatment group and 6.625 cms for the control group. T-test and chi-square were done to determine if there was a significant difference in between groups prior to the initiation of the study. All the variables - age, gender and ulcer size on baseline were found to be not statistically different between the 2 groups **(Table 1)**.

Table 1. Patient Demographics

Subjects (n=32)	Adapalene gel (n=16)	Placebo (n=16)	P-value
Age (mean +/- SD)	55.4375 +/- 14.27096	54.9375 +/- 10.23037	0.7328
Gender (M:F)	13:3	11:5	0.4909
Ulcer size	9.875 +/- 7.99	6.625 +/- 3.324	0.500

Clinical Effects

In the treatment group, 15 (94%) of 16 ulcers demonstrated 50% or greater reduction in the surface area by the end of the study period. On the other hand, only 3 (19%) of 16 ulcers showed reduction of ulcer of 50% or greater. In terms of complete closure, there were 4 (25%) in the adapalene group and 2 (13%) in the placebo group by the end of the study. **Tables 2** and **3** show the summary of change in ulcer size throughout the 16-week treatment period of all patients.

Table 2. Change in ulcer size in the adapalene gel group

Patient	BL	2nd	4th	6th	8th	10th	12th	14th	16th	% Change
1	30	15	8	4	3	3	3	3	3	**90%**
2	19	17	16	10	8	6	3	3	2	**89%**
3	2	1	1	1	*0	0	0	0	0	**100%**
4	15	14	14	14	14	13	8	5	5	**67%**
5	2	3	3	3	4	4	4	4	5	-150%
6	21	20	18	16	16	15	15	6	5	**76%**
7	9	6	6	3	2	1	1	1	1	**89%**
8	2	2	1	1	*0	0	0	0	0	**100%**
9	9	4	4	4	3	3	3	2	2	**78%**
10	11	10	10	9	8	7	6	5	5	**55%**
11	7	6	4	4	4	3	3	3	3	**57%**
12	4	4	4	3	3	2	2	2	2	**50%**
13	4	7	5	1	*0	0	0	0	0	**100%**
14	3	2	1	1	*0	0	0	0	0	**100%**
15	12	12	10	9	5	4	4	3	3	**75%**
16	8	8	7	7	7	6	5	2	1	**88%**

Table 3. Change in ulcer size in the placebo group

Patient	BL	2nd	4th	6th	8th	10th	12th	14th	16th	% Change
1	7	7	7	7	8	8	8	8	8	-14%
2	5	5	5	5	4	4	4	4	4	20%
3	6	6	5	5	5	5	5	5	5	17%
4	4	4	4	4	4	4	4	4	4	0%
5	8	8	7	7	5	5	5	4	4	**50%**
6	7	7	6	6	6	6	6	6	6	14%
7	14	14	13	13	13	12	12	12	11	21%
8	5	5	4	3	2	*0	0	0	0	**100%**
9	2	2	2	2	1	1	*0	0	0	**100%**
10	12	11	12	12	13	13	15	15	15	-25%
11	3	3	3	3	3	3	3	3	3	0%
12	6	6	8	8	8	10	10	10	10	-67%
13	5	4	4	4	4	5	5	6	6	-20%
14	9	9	9	9	9	8	8	7	7	22%
15	10	10	10	10	8	8	8	8	8	20%
16	3	2	2	2	2	2	2	2	2	33%

**Closed completely*

The progress of ulcer healing in both groups with respect to mean surface area and time is shown below (**Figures 2 and 3**).

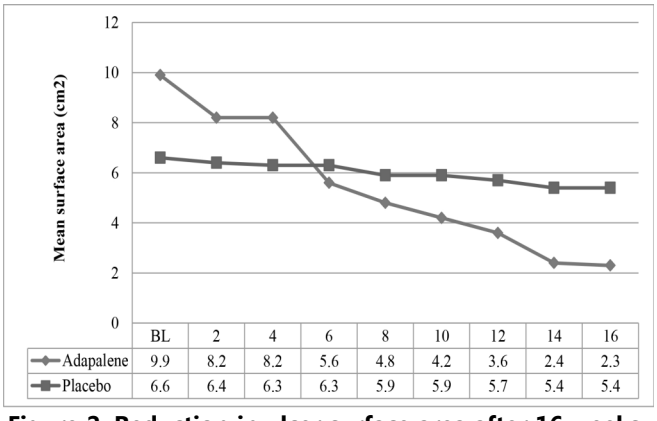

Figure 2. Reduction in ulcer surface area after 16 weeks of treatment

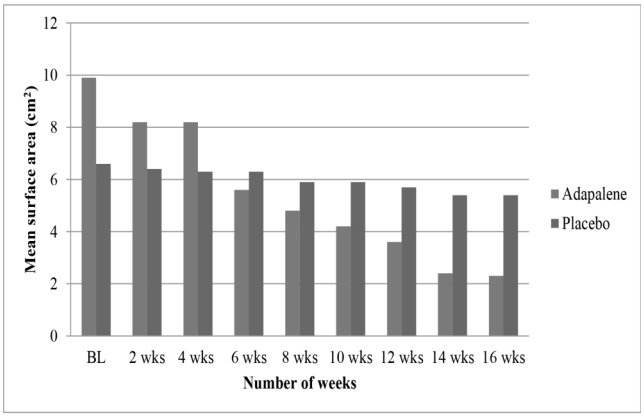

Figure 3. Adapalene group vs. placebo group in the reduction in ulcer

After utilizing ANOVA of repeated measures in the treatment arm, there was a difference in the size of the ulcer from baseline to week 16, as the p value was <0.0005. Since there was a significant difference in the size of the ulcer from the pretreatment to post treatment, a post hoc comparison test was done to assess at which week the size of the ulcer became smaller. The Tukey test determined that on the 12th week, 14th week and 16th week of treatment using adapalene gel, there was a significant difference in ulcer size in between two groups (p values = 0.037, 0.007 and 0.005). Optimal effect of adapalene gel was seen at the 16th week (p = 0.005).

T-test was used to determine if there was difference in the percentage of ulcer surface area after treatment between the treatment and control groups. With a p value of 0.0313, the ulcer size treatment with adapalene gel significantly decreased in comparison with the placebo gel. There were no reported adverse effects such as erythema, pain, nor edema during the 16-week treatment period.

DISCUSSION

This study demonstrated the favorable effect of adapalene 0.1% gel in the closure of neuropathic ulcer among leprosy patients. It showed a more than 50% reduction in almost all the ulcers studied and complete closure at early weeks in a few. There were no adverse effects reported until the end of the study.

Neuropathic ulcer, like most chronic non-healing wounds, result from breakdown of tissue that in turn stimulates the release of enzymes that further enhances connective matrix breakdown and depletion of healing factors. Another feature of these chronic ulcers is an environment containing excessive reactive oxygen species that further damage the cells and healing tissues.

The present study aimed to determine if short -contact application of adapalene 0.1% gel would improve the healing of NU. Through manual planimetry, it was shown that adapalene afforded more than 50% reduction in ulcer surface area, suggesting faster wound healing and granulation tissue formation (**Figures 4 and 5**). Our results also showed that reduction in ulcer size can be observed as early as 12 weeks with optimal results seen at 16 weeks.

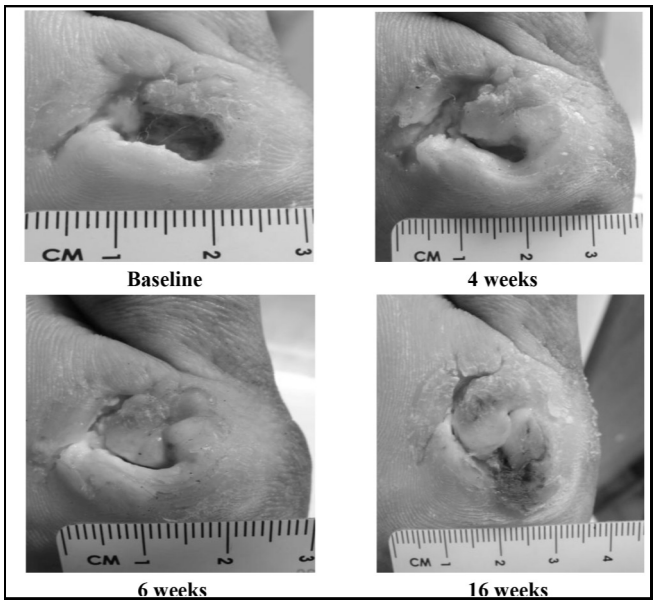

Figure 4. Reduction in ulcer surface area in a representative patient treated with

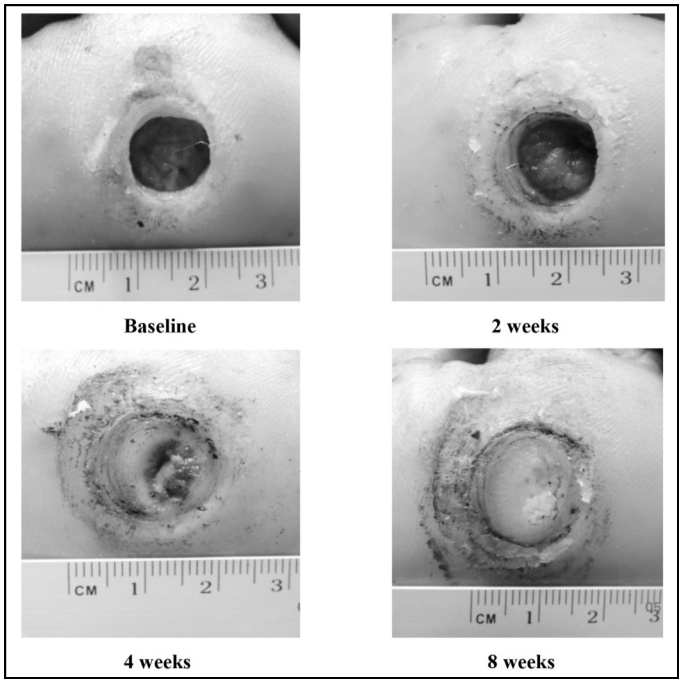

Figure 5. Complete closure of a neuropathic ulcer in another representative patient

Effects of adapalene may be due to the fact that it possesses strong retinoid agonistic properties. It has been reported to produce similar or greater pharmacological activity compared to retinoic acids. Studies on the wound reparative capacity of adapalene reveal that it promotes angiogenesis, enhances collagen synthesis by increasing HP, increases cellular filtration, and stimulates granulation tissue formation. Moreover, collagen, reticular and elastic fibers, which strengthen tension and structural maintenance, are reported to be prominently enhanced.[14] Adapalene is also known to possess anti-inflammatory properties that may be useful in suppressing inflammation associated with chronic non-healing wounds. Its anti-inflammatory action is due to the inhibition of the oxidative metabolism of arachidonic acid via the lipooxygenase pathway. This may contribute to the reduced potential of adapalene to cause erythema and cutaneous irritation.

No adverse effects occurred during the study. However, this is in contrast to the findings in a study done by Basak et al. and Tom et al. which reported more erythema, irritation, and mild pain with tretinoin application.[15,10]

Adapalene is known to behave similarly to tretinoin pharmacologically. This new-generation retinoid addresses many of the problems such as local skin irritation and photoinstability that have limited long-term retinoid use.[17] The biologic effects of retinoids are mediated by two types of receptors: cellular retinoic acid binding protein (CRABP) and nuclear receptors further divided into two: retinoid acid receptors (RARs) and retinoid X receptors (RXR). The development of receptor-specific ligand has been recognized as a way to improve risk-benefit ratio. Adapalene is an example of such as it does not bind to CRABP and has no effect or RXR. The RARs are divided into α, β, and γ subtypes and exhibit distinct affinities for retinoic acid and show characteristic tissue distribution. RAR- γ subtypes are found in the epidermis while RAR- β subtypes are located in the dermis. Tretinoin has a high affinity to all nuclear RARs, whereas adapalene activates RAR- β and RAR- γ only. The unique receptor selectivity of adalapalene offers a clinical advantage over tretinoin, translated into less irritation, more stability, and better tolerance.[18]

Maximum efficacy of a topical drug depends on achieving a high concentration of a drug on the target site while systemic safety relies on minimal passage of the drug to the blood and lymph vessels. Adapalene is characterized by having very low percutaneous absorption once the drug has penetrated the stratum corneum.[18]

One limitation of this study is that ulcer depth was not included in the assessment. As a result, wound healing that may have begun at the ulcer base could not be evaluated and determined.

There is no gold standard in the treatment of NU in leprosy patients. Results were mixed in a systematic review of randomized controlled trials of leprosy with damage to peripheral nerves treated with any measures aimed at healing existing ulcers and prevention of new ones.[6] Eight trials with a total of 557 participants were included. Although three studies that compared zinc tape to more traditional dressings found some benefit, none of these showed a statistically significant effect. One trial indicated that topical ketanserin had a better effect on wound healing than clioquinol cream or zinc paste. Two studies that compared topical phenytoin to saline dressing showed statistically

significant effects in favor of phenytoin for healing of ulcer. Canvas shoes were not much better than polyvinyl chloride (PVC)-boots, and double rocker shoes did not promote healing much more than below-knee plasters.[6] There is an obvious lack of high quality research in the field of ulcer prevention and treatment in leprosy. The success of tretinoin in closure of NU among leprosy patients and diabetic ulcer serves as the basis of selecting adapalene which has similar properties but is more stable, with less adverse effects, less expensive, and better tolerated – features that make it a good alternative in the treatment of foot ulcers in leprosy patients.

CONCLUSION

This randomized controlled study found that compared to placebo, short-contact adapalene 0.1% gel is more effective and generally safe to use in the reduction of surface area of neuropathic ulcer among leprosy patients. It suggests that adapalene may be a novel treatment option for treating neuropathic ulcers.

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