Systemic absorption of 0.1% triamcinolone acetonide as topical application in management of oral lichen planus

Athira Aruna Ramadas, Renju Jose, Arathy SL, Seema Kurup, Marina Lazar Chandy, Sreeja P Kumar

ABSTRACT

Context: Topical corticosteroids are the treatment of choice for oral lichen planus (OLP) due to its potential anti-inflammatory effect. However, chronic nature of OLP often requires long-term and frequent applications, exposing patients to local and systemic side effects.

Aim: To detect the systemic absorption of 0.1% triamcinolone acetonide (TAC) through the oral mucosa of patients with OLP.

Subjects and Methods: This was a pilot pharmacokinetic study carried out in the Department of Oral Medicine and Radiology in collaboration with the Department of Toxicology, over 10 months. A total of twenty patients with OLP were included and advised to apply 0.1% TAC 3 times/day for 2 weeks and 2 times/day for next 2 weeks. Blood samples were obtained on the first and second visits and analyzed for triamcinolone using High pressure liquid chromatography (HPLC).

Statistical Analysis Used: Paired t-test was done to compare visual analog scale (VAS) score for burning sensation at the first and second visits, statistically significant if \( P < 0.05 \). The baseline demographic data were analyzed using descriptive statistics.

Results: Paired t-test was done to compare VAS score for burning sensation at the first and second visits, which turned to being statistically significant \( (P = 0.001) \). Although HPLC is an established method for the detection of TAC, none of the study populations showed evidence of steroid (TAC) in the blood sample during 4 weeks of treatment duration.

Conclusions: 0.1% triamcinolone is a relatively safe drug to be used with no systemic absorption in the standard dose regimen for oral lichen planus.

Key words: High-pressure liquid chromatography, oral lichen planus, topical steroids, transcutaneous absorption, triamcinolone acetonide

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Triamcinolone acetonide (TAC) is a moderately potent corticosteroid commonly used and found to be very effective in treating erosive and ulcerative OLP.\(^8\) There is an increasing trend among dentist and oral physician to use this drug in oral mucosal ulcerative conditions. However, no studies ever tried to measure transmucosal absorption of TAC in human subjects. Hence, the purpose of our study is to detect the systemic absorption of 0.1% TAC through the oral mucosa in OLP patients by looking for the presence of TAC in serum samples of patients after 4 weeks of treatment.

**SUBJECTS AND METHODS**

This was a pilot pharmacokinetic study carried out in the Department of Oral Medicine and Radiology in collaboration with the Department of Toxicology, over 10 months, on subjects reporting to the Dental Outpatient Department. Ethical clearance was acquired from the Institutional Ethical Committee before the onset of the study. At the time of screening, patients were explained regarding the study and informed consent was obtained from those interested in participation.

**Study population**

A total of twenty patients with clinical signs and symptoms of OLP histopathologically confirmed by incisional biopsy were included. The age group ranges from 20 to 60 years and ensures that they did not undergo any treatment for the present condition. Exclusion criteria include pregnancy, lactation, long-term corticosteroid therapy, other mucocutaneous disorders, cutaneous lichen planus with oral lesions, and patient prior history of medication for lichen planus.

The first visit consisted of collecting the demographic data, general history, medical history, personal history, history of patient’s experiences with the lesions, and clinical examination. Pain intensity was scored using a visual analog scale (VAS) of 0–10 (with 1 mm division, where “0” is no pain and “10” is worst possible pain). Other data recorded were sites of involvement, lichen planus scoring, and duration of a lesion. The clinical data were scored according to the criteria used by Thongprasom et al. [Table 1].\(^9\)

An incisional biopsy was carried out in the first visit and histopathologically confirmed lichen planus patient constituted the study population. The medication was initiated 10 days after biopsy. VAS score and clinical scoring were also recorded at 4th week after topical steroid therapy.

**Study medication**

Study medication was Tess Buccal Paste (Troikaa Pharmaceuticals Limited. Ahmedabad, India), and each gram of it contains 1 mg TAC in an emollient dental paste containing carboxymethylcellulose sodium, gelatin, and pectin in a plasticized hydrocarbon gel (a polyethylene and mineral oil gel base). All participants were supplied with 0.1% TAC, and they were trained how to apply one fingertip unit of the drug on mucosal surfaces (previously dried with a gauze), avoiding swallowing for some minutes, and trying not to drink and eat for at least 1 h. They were prescribed to apply above medication 3 times a day for 2 weeks and 2 times a day for the next 2 weeks. Blood samples were collected from each patient before the first application of drug (control sample) and on the 4th week after application of drug (test sample) as prescribed. Inquiry regarding the drug application was done over telephonic conversation on an alternative day basis. Patients who were not consistent with the treatment regimen were excluded from the study population. Subjects were enquired regarding any adverse effects occurred during the study period on the second visit and recorded.

**Blood sample collection**

Blood samples were collected from study participants by venal puncture in vacutainer (red top). Samples were stored at −20°C until analysis and protected from light.

**Instrumentation**

**HPLC**

The high-performance liquid chromatography of Shimadzu Prominence I LC2010 consists of photodiode array detector, calibrated analytical balance accurate to ±0.01 mg (Sartorius), vortex mixer, sonicator - with temperature control, centrifuge, volumetric flasks, and pipettes of Class A-assorted size.

**Chemicals and reagents**

This being a preliminary pilot study, we had used commercially available triamcinolone and prednisolone injection preparations as the reference standard compounds. HPLC-grade methanol and HPLC-grade acetonitrile were procured from Qualigens Fine Chemicals Ltd. The water used was of Millipore water.

**Standard and sample solutions**

**Standard solutions**

The drug samples of TAC and prednisolone internal standard (IS) were considered as stock solutions of reference standards and from that the solutions of subsequent concentrations were prepared by diluting. Working standards were prepared using mobile phase. Stock solutions were stored at −20°C until analysis and protected from light.

**Table 1: Thongprasom et al. scoring criteria for lichen planus**

| Score | Description |
|-------|-------------|
| 0     | No lesion, normal mucosa |
| 1     | Mild white striae, no erythematous area |
| 2     | White striae with atrophic area <1 cm |
| 3     | White striae with atrophic area >1 cm |
| 4     | White striae with erosive area <1 cm |
| 5     | White striae with erosive area >1 cm |

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\(^{8}\) Ramadas, et al. 2016

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\(^{9}\) Thongprasom et al. 2016: scoring criteria for lichen planus
Systemic absorption of topical corticosteroid

Sample preparation
A 25 µL (5 µg/mL) of IS was added to 100 µL serum sample and vortex-mixed for 30 s. Then, 175 µL acetonitrile was added and mixed on a vortex mixer for 2 min. After centrifugation (5000 rpm, 10 min), 150 µL supernatant was transferred to a micro vial and 50 µL of the solution was injected onto the column.

Sample analysis
In the given study, the separations of TAC and IS achieved by a reversed phase column (Spinco Biotech C18 (2) 250 mm × 4.6 mm, 5 µm.) at 35°C. The mobile phase consisted of 60:40 (methanol: water, v/v) at a flow rate of 1.5 mL/min. Ultraviolet detection was performed at 250 nm. The column temperature was 35°C. Run time was fixed as 20 min, and the automatic injector filtered at 50 µL was set. Under optimized conditions, TAC and IS accurately resolved from baseline and separated. All the injections/runs were done in a triplet for quality assurance, i.e., both control and test samples. All the chromatographs were recorded by using LabSolutions Labsolution Lite software, Shimadzu corporation, Japan.

Statistical analysis
The baseline demographic data and lichen planus history were summarized with descriptive statistics. Effectiveness of TAC in the lichen planus symptoms was analyzed using paired t-test (using SPSS 20, IBM, Armonk, NY, United States of America).

RESULTS

Clinical
A total of 20 histologically confirmed lichen planus patients were enrolled. There were 11 (55%) females and 9 (45%) males. The age varied from 29 to 62 years (mean ± standard deviation [SD] = 47.05 ± 8.52) [Tables 2 and 3]. Fifty percent of patients were in the age group of 40–50 years [Table 3]. Mean duration of a lesion was 4.05 with an SD of ± 2.5. Average VAS score at baseline was 5.5 [Table 2]. Seventy-five percent of a patient had a lesion over the buccal mucosa [Table 2]. According to Thongprasom lichen planus scoring, at baseline, 50% had Score 1, 35% had Score 2; 15% had Score 3 [Table 4]. At 4 weeks after application of triamcinolone, 75% had Score 1; 20% had Score 2, and 5% had Score 3. The average VAS score at 4th week was 2.1 [Table 5] which was statistically significant compared to the first visit (P = 0.001). The above data show the effectiveness of TAC on lichen planus. No adverse effects were reported.

Experimental

Triamcinolone acetonide in blood samples by HPLC
Before starting the study, we tried running the TAC at different concentration to find out the minimum concentration [Figure 1]. Thus, to obtain baseline information, the test samples and control sample of blood were analyzed for TAC.

DISCUSSION
Over a past 50 years, corticosteroid was the main choice in clinical usage for various disorders ranging from inflammatory to immune mediated. Mechanism involved in the suppression of inflammation by corticosteroid

Table 2: Demographic data

| Variable                  | Mean±SD  |
|---------------------------|----------|
| Age (years)               | 47.05±8.52 |
| Duration (months)         | 4.05±2.8  |
| VAS score                 | 5.5±2.1  |

| Variable                  | N (%)    |
|---------------------------|----------|
| Female                    | 11 (55)  |
| Male                      | 9 (45)   |
| Location                  |          |
| Buccal mucosa             | 15 (75)  |
| Buccal mucosa + tongue    | 04 (20)  |
| Buccal mucosa + gingiva   | 01 (5)   |
| Medical status            |          |
| Hypertensive on medication| 4 (20)   |
| Diabetes mellitus on medication| 1 (5) |
| Deleterious habits        |          |
| Smoking                   | 3 (15)   |
| Alcoholism                | 1 (10)   |

SD=Standard deviation, VAS=Visual analog scale

Table 3: Age distribution among study population

| Age group | n (%) |
|-----------|-------|
| 20-30     | 2 (10) |
| 31-40     | 6 (30) |
| 41-50     | 10 (50) |
| 51-60     | 4 (20) |
| 61-70     | 2 (10) |
| Total     | 20 (100) |

Table 4: Scoring of oral lichen planus according to Thongprasom at baseline and 4th week after topical application

| Thongprasom sign scoring | n (%) at baseline | n (%) after topical application |
|--------------------------|-------------------|---------------------------------|
| Score 1                  | 10 (50)           | 15 (75)                         |
| Score 2                  | 7 (35)            | 4 (20)                          |
| Score 3                  | 3 (15)            | 1 (5)                           |

Table 5: Visual analog scale score at baseline and 4 weeks after application of medication

| VAS score at baseline | VAS score at 4 weeks after application | P     |
|-----------------------|---------------------------------------|-------|
| 5.5±2.1               | 2.10±1.56                             | 0.001*|

*Paired t-test, significant if P<0.05. SD=Standard deviation, VAS=Visual analog scale

Under optimized conditions, TAC and IS accurately resolved from baseline and separated. Retention time of drug samples of TAC and IS found to be 3.8626 ± 0.0244 min and 2.48 ± 0.26587 min, respectively. All the injections/runs were done in a triplet for quality assurance. Both control and test samples did not show specific peek for TAC while all of them showed a peak for IS [Figure 2].
includes reduction of the exudation of leukocytes and plasma constituents, maintenance of cellular membrane integrity, inhibition of lysosome release from granulocytes, inhibition of phagocytosis, stabilization of the membranes of the intracellular lysozymes and inhibits proliferation of fibroblast.\[10\]

Corticosteroids have many side effects and should always be used with caution, especially in medically compromised patients. To decrease various side effects, the basic rule for using a corticosteroid is to use a topical of strength that adapts to the seriousness of the clinical symptoms, prescribed at the lowest concentration possible.\[8\] Topical corticosteroids are classified according to their efficacy into four potency classes, where Class I represent mildly potent, Class II moderately potent, Class III potent, and Class IV very potent corticosteroids.\[11\] However, at present, the low potency steroid drugs are available as over the counter. There is also a tendency among dentist, physician to prescribe topical steroids for any oral ulcer of unknown etiology.

Lichen planus is a common chronic immunological mucocutaneous disorder of stratified squamous epithelium encountered in dental scenario. Different clinical manifestations include papular, reticular, plaque, erosive, atrophic, and bullous form, depending on the degree of inflammatory response within the connective tissue. It is a result of abnormal T-cell-mediated autoimmune response to basal epithelial cells.\[12,13\] It affects approximately 1–2% of the population globally and in India, the prevalence ranges from 0.1% to 1.5%.\[2,9\] OLP predominantly affects women over 40 years, which was consistent with our study.\[14\] Corticosteroids are the mainstay of medical treatment of OLP because of their dampening effect on cell-mediated immunity, thereby modulating the immune function. Routes of administration include topically,
intralesional, or systemically.\textsuperscript{[10,12]} Topical steroids are as effective as systemic corticosteroids with fewer side effects. Chronic nature of disease demand's long-term steroidal therapy, which may induce drug tolerance, adrenal insufficiency, pseudomembranous candidiasis, mucocutaneous atrophy and Cushing's syndrome, blanching of mucosa, hypopigmentation, delayed wound healing, and increased friability of mucosa.\textsuperscript{[8,15,16]}

The stratum corneum acts as the rate-limiting barrier to percutaneous drug absorption. Thickness of this layer varies at different body parts; thus, drug penetration also varies at different sites being the highest through the mucous membrane and scrotal skin and the least through palmoplantar skin.\textsuperscript{[4,5]} Absorption also depends on drug concentration, formulation, and times of application. It is estimated that permeability of oral mucosa is 4–4000 times greater than that of skin. Permeability in descending order is sublingual > buccal > palatal; this is based on relative thickness of the epithelium. It is currently believed that the permeability barrier in the oral mucosa is a result of intercellular material derived from the so-called “membrane coating granules.” This barrier exists in the outermost 200 \( \mu \)m of the superficial layer. Once this layer has been modified or removed, which leads to increase systemic absorption.\textsuperscript{[17]} This layer undergoes modification in lichen planus.

Hence, the purpose of our study was to analyze the systemic absorption of topical triamcinolone, a moderately potent corticosteroid in the OLP patients. A comprehensive literature search revealed that there are several methods for the detection of triamcinolone in different sample types. Most of the methods for detection of triamcinolone are hugely expensive involving sample extraction cartridges, etc.\textsuperscript{[18]} The method choose is an adaptation of the method validated by Nemutlu et al.\textsuperscript{[18]} This being a pilot study, we aimed for the qualitative detection of triamcinolone in the blood from the patients who are under triamcinolone therapy. We came to decide on this method for our study by prior evaluating the method with serum samples properly spiked with TAC.

Even though erosive, atrophic and bullous forms of lichen planus can be assumed to have systemic absorption due to alteration in epithelium, facilitating more absorption our samples did not show any systemic absorption.

In our study, ten patients had reticular lichen planus and ten had atrophic lichen planus. However, by HPLC, both control and test samples did not show a specific peak for TAC while all of them showed the peak for IS. One possible explanation for this phenomenon may be the small dosage of triamcinolone which made detection demanding as we were applying the drug in tapering dose. However, it is unlikely that the steroid would have passed through the column undetected even in the smallest concentrations because of the extremely high sensitivity of the HPLC system, which allows for measurements in picograms. Another reason may be due to the complex environment of oral cavity, which limits the absorption of the drug. This includes the degree of keratinization, surface area available for absorption, mucus layer of salivary pellicle, intercellular lipids of the epithelium; basement membrane, and lamina propria. In addition, the absorptive membrane thickness, blood supply/lymph drainage, cell renewal, and enzyme content will all contribute to reduce the rate and amount of drug entering the systemic circulation.\textsuperscript{[17]} The time duration of 4 weeks was enough to detect the cumulative systemic absorption because <2% of topically applied steroid is absorbed into systemic circulation after a single stay on application of more than 1 day.\textsuperscript{[14]} Further, studies indicate that adrenal suppression becomes evident as soon as 24 h after initiating topical steroid therapy.

Plemons et al. conducted a study in 10 patients with erosive lichen planus and eight control patients to investigate the absorption of topical steroid fluocinonide from blood samples at baseline, day 3, and day 21 using HPLC. They concluded that none of the patients showed evidence of steroid during the study.\textsuperscript{[3]} Varoni et al. in their study evaluated the transmucosal assimilation of clobetasol after its application on oral mucosa. Blood samples were collected from 10 patients with oral inflammatory conditions (OLP and Mucous membrane pemphigoid (MMP)) who were regularly applying 0.05% clobetasol propionate and 14 healthy volunteer patients. They analyzed sample using ultra-performance liquid chromatography and concluded certain amount of accumulation.\textsuperscript{[7]}

In our study, TAC was effective in alleviating the signs and symptoms of lichen planus. Further studies were to be done on a large population with long follow-up duration. There are several limitations to our study; being a pilot study, we included only small number of population. Even though emphasis was placed on proper application, patient compliance was one of the limitations and patients who were not consistent with treatment were excluded from study population. The standard used for HPLC was not according to USP grade reference standard. Although HPLC was an established method for detection of triamcinolone from various body fluids, we were not able to detect triamcinolone from blood (serum) in the study population after application for 4-week duration. For an accurate determination of total steroid TAC concentrations in serum and other samples with complex matrices, samples may need to be extracted before assaying. Extraction eliminates potential interfering substances, such as bulk proteins and lipids. Extraction may also be necessary to concentrate the sample to within the assay’s measurement range. We had used traditional liquid–liquid extraction method for our study. Any purification technique, recovery of the desired substance is likely to be incomplete. For better efficiency,
a solid phase extraction method may be used. This study is open for researchers to have further experiments on the same.

CONCLUSION

0.1% of TAC is a relatively safe drug to be used with no systemic absorption in the standard dose regimen for OLP.

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Conflicts of interest
There are no conflicts of interest.

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