

The efficacy of topical hyaluronic acid in the management of recurrent aphthous ulceration

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BACKGROUND: The aim of this study was to evaluate the efficacy of a topical hyaluronic acid (HA) preparation (0.2%) in the management of recurrent aphthous ulceration (RAU).

METHODS: One hundred and twenty patients with RAU participated in a randomized, placebo controlled, double-blind trial to evaluate the efficacy of the topical HA and preparation. Outcome measures include soreness relief on immediate application (recorded over 60 min). Thereafter, patients completed a log diary recording soreness from the ulcers, occurrence of new ulcers and ulcer duration.

RESULTS: Both topical HA and placebo resulted in a significant reduction in ulcer soreness following immediate application ($P = 0.0004$). Throughout the rest of the investigation period, there was no significant differences ($P > 0.05$) between the treatments for reducing soreness. Patients treated with topical HA recorded few ulcers on day 5 of the investigation than those treated with placebo ($P < 0.001$). Likewise, the occurrence of new ulcers was lower in the HA treated group on day 4 when compared with placebo ($P = 0.047$).

CONCLUSION: Topical HA (0.2%) may be of benefit in the management of RAU. Immediate reduction of symptoms appears to be a barrier effect.

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Introduction

Recurrent aphthous ulceration (RAU) is a common inflammatory condition of unknown aetiology, although a variety of predisposing and other risk factors have been identified. It is the most common form of oral ulceration and approximately 20% of the population will suffer from RAU at some time in their lives (1)

Topical preparations appear to be the main agents used in the treatments of RAU, especially those with an anti-inflammatory action. However, for such agents to be effective, they should be easy to apply and retained at the site of ulceration for as long as possible. The active ingredient needs to be released from the delivery vehicle and exhibit substantivity.

Hyaluronic acid (HA) is a linear polymer of glucuronic acid N-acetylglucosamine disaccharide. Most cells have the capacity to synthesis HA during some point of their cell cycle. The main function of HA appears to be in tissue healing. In this process, HA is implicated in a range of activities including activation and moderation of the inflammatory responses, promoting cell proliferation, migration and angiogenesis, promoting re-epithelization via proliferation of basal keratinocytes and reducing collagen disposition and scarring (2). Animal studies have shown that HA can promote healing in a variety of tissues (2). Clinical studies have shown that topical application of HA promotes healing of both venous leg ulcers (3), and the nasal mucosa after surgery (4). It also has been shown to reduce the incidence of high-grade radio-epithelitis in patients who have undergone radiotherapy for head and neck, breast or pelvic carcinomas (5). A hyaluronic preparation is available commercially, but its usefulness for the management of RAU has not been proved. The aim of the present study was to carry out randomized, placebo controlled investigation into the efficiency of a topical HA gel 0.2% (RF02 APH) in the relief of symptoms of RAU.

Materials and method

One hundred and twenty adult patients who presented with RAU participated in the study. All patients underwent a full haematological screening before entering the study. The parameters measured included FBC, serum B12, red cell folate, serum ferritin and endomysial antibody. Only patients whose values were within the normal range were included in the study. Other entry criteria included a clear history of RAU occurring at least twice a year and to have at least one ulcer present prior to dosing. Patients were excluded if they

exhibited any underlying haematological disorder, taking non-steroidal anti-inflammatory drugs (NSAIDs), immunosuppressants, other anti-inflammatory agents or chemotherapeutic drugs, suffering from an uncorrected dietary defect, or had a history of probable sensitivity to mouthwash or toothpaste.

The protocol for the study had received approval from the local joint Ethics Committee. Patients for the study were enrolled from the Oral Medicine Clinic, Newcastle Dental Hospital. Eligible patients had to present with discomfort arising from an ulcer. For these patients a topical application of HA gel 0.2% or identical placebo was applied by a Clinician to the ulcerated area. Allocation of the gel to the patient population was randomized and double-blind. Patients were instructed how to apply gel for subsequent applications.

Following first dosing, patients were retained in the clinic and asked to record on 100 mm visual analogue scales (VAS) the discomfort arising from the ulcerated area. The boundaries of the scale were marked 'no soreness' and 'worst possible soreness'. Recordings were made at baseline (before gel application) and at 5, 10, 15, 20, 30, 45, 60, 120, 180 and 240 min after dosing. The first 60 min of the recording were supervised and the remaining observations were carried out on a log diary provided to the patients on discharge. On completion of the first 60 min, patients were given a sufficient supply of gel to apply two to three times per day for the next 7 days. Patients were instructed to apply the gel after breakfast and after their evening meal and at one other time if desired. Times of gel application were recorded in the log diaries. Further discomfort recordings were made 1 h after application for 7 days. In addition to recording discomfort, patients were also asked to record number of ulcers present in their mouth and the occurrence of any new ulcers during the treatment period. Completed log diaries were reviewed at a clinical appointment on day 8 and any remaining gel returned. At this appointment, patients were asked to make an overall assessment of the gel on 5-point description scale (very poor, poor, moderate, good and very good). Patients were also asked whether they would use the gel again in the management of their RAU.

Statistical analysis

The main outcome measure of this study was the relief of soreness based upon repeated VAS recording. These scales have been used extensively in the measurement of pain and other subjective responses, but have not been utilized in the assessment of therapies for the treatment of RAU. The power calculation for this study was therefore based upon observed standard deviations from analgesic efficiency studies. Assuming a standard deviation of 15 mm on the 100 mm VAS, a power calculation based upon a sample size of 60 patients, per group and alpha level of 0.05 would allow the detection of a mean difference between treatments of 10 mm on the VAS with 84% power.

Analysis of variance according to the model of repeated measurements within-between subjects, integ-

rated by covariate analysis at the basal time was used to assess differences between treatment groups for soreness scores and ulcer history (number of ulcers present in the mouth each day and number of new ulcers). A Pearson chi-square test was used to assess differences between treatment groups for the distribution of patients' scores for their overall assessment of the medication. *P*-value < 0.05 was considered statistically significant.

Results

A total of 120 patients were enrolled into the study and completed the first supervised part of the investigation and returned their log diaries. Four log diaries were subsequently rejected because of protocol violations. Of the remaining 116 patients, 60 received HA 0.2% and the remainder placebo gel. Demographic details of patients together with details of their baseline ulcer history and soreness scores are shown in Table 1. The number of ulcers and baseline soreness scores were similar for the two treatment groups (*P* > 0.05).

Following initial application, patients in both treatment groups reported a rapid reduction (*P* = 0.0004) in their discomfort scores arising for their ulcers (Fig. 1). This level of reduction was sustained for both treatment groups for about 30 min. There after scores started to return to baseline. A similar position was observed for the subsequent 3 h, and throughout the rest of the 7 day observation period (data not shown). The number of

Table 1 Demographic details of patients and baseline ulcer study for those who participated in study

	Placebo	HA 0.2%
Total number	60	60
Male	17	18
Female	43	42
Average age	36.65	37.05
Ethnic origin	58 White 2 Asian	58 White 2 Asian
Protocol violators	3	1
Mean baseline soreness scores (mm) as recorded on 100 mm VAS	52.28	42.03
Average number of ulcers at baseline	2.51	1.95

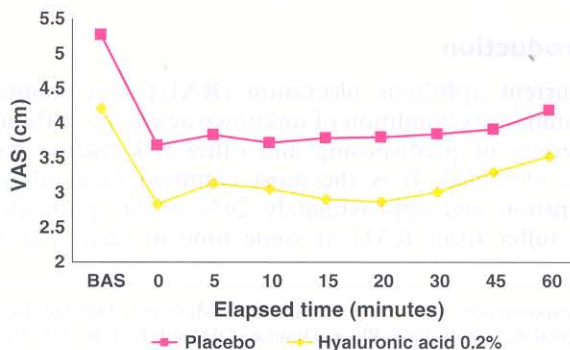


Figure 1 VAS Scores Post-Gel Application.

Table 2 Mean number of ulcers (\pm SEM) for each treatment group during the 7 day investigation period

Time (days)	Placebo	HA 0.2%	Significance between groups
Baseline	2.5 \pm 0.24	1.96 \pm 0.24	0.12
Day 2	2.7 \pm 0.25	2.2 \pm 0.25	0.16
Day 3	2.58 \pm 0.25	2.13 \pm 0.25	0.21
Day 4	2.48 \pm 0.25	1.88 \pm 0.25	0.09
Day 5	2.4 \pm 0.26	1.65 \pm 0.25	0.04
Day 6	2.2 \pm 0.28	1.56 \pm 0.28	0.11
Day 7	2.0 \pm 0.28	1.53 \pm 0.28	0.22

Table 3 Ulcer history during 7-day investigation period for patients treated with hyaluronic acid and placebo

Ulcer count at baseline	Number of patients	Number of patients free from ulcers
(a) Hyaluronic acid		
1	37	19
2	10	3
3	5	1
4	2	0
5	2	0
6	2	0
8	2	1
Total	60	24
(b) Placebo		
1	28	13
2	11	3
3	7	1
4	5	1
5	1	1
6	5	0
7	2	0
10	1	0
Total	60	19

ulcers before medication was similar for both treatment groups (Table 2). Patients were asked to record each day the number of ulcers present in their mouth and mean number of ulcers for each treatment is shown in Table 2. There was a slight decline in the number of ulcers, irrespective of treatments, over the 7 day observation period. However on day 5 patients in the RF02APH (study compound) group had significantly fewer ulcers than those treated with placebo. More details of ulcer history with respect to number of ulcers per patient at baseline compound to number of patients free from ulcers after 7-days of treatment is shown in Table 3a and b. Although there is a significant decline in both treatment groups ($P < 0.001$), there was no difference in ulcer history between treatments.

In both treatment groups, new ulcers occurred throughout the investigation period. On day 4 the incidence of new ulcer occurrence was significantly lower in the RF02APH (active) group when compared with placebo treatment patients ($P = 0.047$). For the other days, the new ulcer occurrence rate was similar (Table 4).

Patients overall assessment of their treatments is shown in Table 5. There was no significant difference

Table 4 Number of patients with ulcer occurrence during 7-day investigation period

Day	Placebo	HA 0.2%
1	16	12
2	5	5
3	5	1
4	10*	2
5	7	7
6	5	2
Total	48	29

*Significant difference between treatment groups $P = 0.047$.

Table 5 Distribution of scores from patients overall assessment of their treatment

Score	Placebo	HA 0.2%
Very good	10	17
Good	11	15
Moderate	17	12
Poor	12	10
Very poor	7	5
Not recorded	3	1

($P = 0.075$) between treatments in the distribution of scores. Unwanted effects were few and showed no difference between treatment groups.

Discussion

Topical medications appear to be the first choice treatment for RAU. Such preparations do have limitations with respect to drug delivery, subsequent compliance and retention on the oral mucosa. These features probably impact significantly on the efficacy of the agent, but do present challenges to the pharmaceutical industry for appropriate development.

Parameters used to evaluate the outcome of treatments in the management of oral ulceration include 'ulcer days index' (number of days free from ulcers), incidence of ulceration, duration of ulceration, severity of pain and user preference (6). The 'ulcer day index' is the sum of the number of ulcers each day over a period, usually 4–8 weeks. It indicates the severity of the episode and reflects the mean prevalence and duration of ulcers as well as the number of ulcer-free days in a specific period. The incidence of ulceration is the number of new ulcers forming within a specified period, usually a period of no less <4 weeks. The duration of ulceration is the mean duration of individual ulcers. Pain can be assessed subjectively by patients on pain scores. User preference allows the patient to subjectively indicate the acceptability of a particular product.

The most significant outcome of this study was the immediate and sustained reduction in pain scores after application of HA 0.2% and placebo. Both preparations (RF02APH and placebo) were based on the same formulation with the only exception of HA, substituted, in the latter by inert material) caused a significant immediate reduction in discomfort following applica-

tion. This would suggest some protective or barrier function arising from placement of this specific gel. The effects seemed to last for at least 30 min and there was no difference in efficacy between treatment groups. This protective or barrier for property arising from the gel may support further the use of topical medications in the management of symptoms arising from RAU.

We also observed a reduction in the number of ulcers over time in the HA treated group. This was observed on day 5 and would imply that exogenous high molecular weight HA is promoting healing when compared with placebo treatment. Indeed this is a major physiological property of HA. HA was only applied topically in this study, thus the physical chemical properties are important in relation to efficacy. HA is a hygroscopic macromolecule and solutions are highly osmotic. In the skin and perhaps on the oral mucosa, this property is likely to be relevant in controlling tissue hydration during periods of change such as the inflammatory process or response to tissue injury. This is also particular relevance for cell proliferation and migration, when HA synthesis contributes to local foci of tissue hydration. This results in the weakening of cell anchorage to the extra cellular matrix, allowing temporary detachment to facilitate cell migration and division (7). In the hydrated state, much of the water around the HA molecule is immobilized which results in restriction of movement of water and small molecules (8). The highly viscous native of HA also contributes to retardation of viral and bacterial passage through the HA-rich pericellular zone (9, 10). In inflammation, HA may also have a moderating effect through free-radical scavenging (11, 12), antioxidant effect (13), as well as through exclusion of tissue degrading enzymes from the immediate cellular environment and from other structural components of the extra cellular matrix (14). All of these properties are likely to contribute to the healing process and may account for the reduction in the ulcers found in the treatment group at day 5. Some of these properties may also account for the reduction in the occurrence rate also observed in the active treatment group on day 4.

This double blind randomized controlled trial looks particularly at the efficacy of HA 0.2% in the management pain associated with RAU as well as measuring the patients' overall acceptability of the product. We also made observations on the possible effect of HA 0.2% on ulcer duration, although the time period over which the ulcers were recorded was too short to make accurate duration measurements. Other studies on the effect of topical preparations on the management of RAU use a variety of different parameters outlined above. This, therefore, makes direct comparison between HA and other topical preparation difficult. Nevertheless, pain scores are commonly used, so some comparisons can be made.

The effect of chlorhexidine gluconate mouth rinses on RAU have been studied and a recent review of these studies (15) concluded that chlorhexidine mouthwash did not influence the incidence of mouth ulcers, but that it reduces the severity of each episode of ulceration. Evidence for this conclusion has come from three

randomized clinical trials of crossover design (16–18). Overall, chlorhexidine appears to play a role in the management of aphthous ulceration, possibly by reducing the prevalence of secondary infection, but it does not provide significant immediate pain relief.

Topical steroids are commonly used in the management of RAU. Only one crossover, randomized controlled trial demonstrated a significant reduction in pain compared with placebo, but showed no effect on reducing the frequency of RAU occurrence (19). The remaining studies give some weak evidence of a reduction in pain and ulcer duration, without significant adverse effects (20–25). It was also reported that most users preferred topical steroids to control preparations (21–24). The evidence, therefore suggests that topical steroids are of value to this group of patients. Nevertheless, HA 0.2% offers advantages over steroids in that it is safe in all patients including infants and pregnant women, in whom there may be reluctance to use steroids.

Amlexanox 5% is a further topical agent used in the management of RAU. This agent possesses both anti-inflammatory and anti-allergic properties. Results from various clinical trials have demonstrated that amlexanox facilitates the healing of oral ulcers by reducing their duration by up to 2 days (26), accelerates the resolution of ulcer pain and healing (27, 28). A recent study has also shown that early application of amlexanox in the prodromal stage of RAU does appear to abort an outbreak (29).

This product, therefore, is of value in the overall management of RAU, particularly if applied at very early stages. HA 0.2% can be applied at any stage of ulceration and provides immediate reduction in pain levels, thereby offering a different therapeutic approach to patients.

It would appear therefore, that chlorhexidine can reduce the duration of ulcers, but can cause some discomfort to such patients on initial application. Amlexanox (5%) hastens the healing process of ulcers and the duration to complete pain relief. Topical steroids help reduce the duration of ulcers, but provide little pain relief. Additionally, although the risk of steroid complications is low if used for a limited period of time and used correctly, topical steroids cannot be used in all patients. HA 0.5% provides immediate pain relief on application regardless of the stage of ulceration. It can be used in all individual including infants and pregnant women without risk of complications or drug interactions. There is no risk of overdose and can be safely recommended to individuals who may not follow instructions easily. It is widely available as an over the counter preparation and does not cause any discomfort, making it acceptable to children. In addition, it would appear to accelerate healing, although further studies are recommended to evaluate this property.

Topical applications of HA 0.2% does appear to be of benefit in the management of RAU. Immediate application reduces discomfort but this is purely a barrier or protective mechanism from stimuli arising in the oral environment. HA 0.2% may be of benefit in promoting

healing of RAU as reflected in ulcer duration. There is also a slightly lower risk of occurrence rate when patients are treated with this preparation. HA 0.2% is a new topical agent available for treatment of RAU. It has few unwanted effects and its main benefit is in facilitating healing of this common oral condition.

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