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Formulation and clinical evaluation of a hydrocortisone solution for the treatment of oral disease

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Abstract

The purpose of this study was the formulation and clinical evaluation of a steroid solution for the treatment of oral disease. The steroid used for this work was hydrocortisone and its solubility was increased by complex formation with 2-hydroxypropyl-β-cyclodextrin. In a clinical efficacy study of the hydrocortisone solution in 50 patients, there were no reports of ill effects and no side effects such as erythema or superinfection were noticed. Of those treated, 78% showed improvement, with 48% showing considerable clinical improvement. The preliminary clinical observations show that this hydrocortisone solution was easy to use, had a clinical efficacy that compares well with much more potent preparations and thus should help to minimise the prevalence of harmful side effects of the treatment of these distressing conditions.

Keywords: Oral disease; Hydrocortisone; 2-Hydroxypropyl-β-cyclodextrin; Clinical evaluation

1. Introduction

Topical steroids have been recommended for the treatment of several diseases of the oral mucosa including recurrent (aphthous) ulceration, erosive lichen planus, benign mucous membrane pemphigoid and desquamative gingivitis (Zegarelli, 1983; Oliver and Winkelmann, 1993; Bottomley and Rosenberg, 1993; Seymour, 1994). For many years, the only commercially available steroid preparations for topical use in the mouth have been in the form of paste or gel. This is rather impracticable for treating extensive ulceration, erosion or inflammation of the oral mucosa. A mouthwash containing steroid could be a useful alternative to topical orabase (Zegarelli, 1991) but the inherent insolubility and instability of otherwise suitable steroids for mouthwashes have hin-

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dered their commercial preparation. Some clinicians have resorted to mouthwashes prepared directly by the patient using betamethasone tablets dissolved in water or mouthwashes using hydrocortisone prepared for parenteral administration.

The aim of the present study was to develop a steroid mouthwash that would withstand manufacturing processes and which would then conform to requirements for drug stability and preservative efficacy. Potential benefits of such a mouthwash include ease of application to all areas of the oral mucosa and a lower incidence of side-effects compared with systemic steroids. The mouthwash developed in this study underwent preliminary evaluation for clinical efficacy in a trial of 50 volunteer patients.

Drawbacks to formulating a steroid mouthwash include the low solubility of many steroids in water, instability of steroids at neutral pH and the bad taste of most steroids. The steroid chosen for this study, hydrocortisone, is sparingly soluble in water and its stability is affected by pH but it has previously been shown that both solubility and stability of hydrocortisone can be improved by forming an inclusion complex of the drug with cyclodextrin (Møllgaard Andersen and Bundgaard, 1983). Cyclodextrins are cyclic oligosaccharides with hydroxyl groups on the outer surface and a void cavity in the centre (Szejtli, 1988). The most common cyclodextrins are α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin consisting of 6, 7 and 8 α1,4-linked glucose units, respectively. Cyclodextrins are capable of forming inclusion complexes with a wide variety of hydrophobic molecules by taking up a whole molecule, or some part of it, into the cavity (Pitha et al., 1986). Incapsulation of a drug molecule will affect many of its physicochemical properties. The cyclodextrin most useful for incorporating drugs is β-cyclodextrin which has a limited solubility in water, however, alkylation or hydroxyalkylation increases the solubility of the compound (Yoshida et al., 1988). β-cyclodextrin derivatives have been shown to possess similar solubilizing and stabilizing effects on drugs in aqueous solutions as the parent β-cyclodextrin (Loftsson et al., 1991a). In this work, the cyclodextrin derivative used was 2-hydroxypropyl-β-cyclodextrin, which forms noncovalent inclusion complexes with hydrocortisone, increasing its solubility and stability in aqueous solutions.

2. Materials and methods

2.1. Materials

Hydrocortisone was obtained from Apodan (Denmark) and 2-hydroxypropyl-β-cyclodextrin from Wackers Chemie (Munich, Germany). All other chemicals used were commercially available products of special reagent grade.

2.2. Preparation of solutions

Hydrocortisone (0.30% w/v) was dissolved in a 4.5% (w/v) 2-hydroxypropyl-β-cyclodextrin solution by heating. Hydroxypropylmethylcellulose (0.5% w/v) was used to increase the viscosity of the solution. Solutions were preserved using (a) 0.1% (w/v) methyl parahydroxybenzoate, (b) 0.01% (w/v) benzalkonium chloride and 0.05% (w/v) sodium edetate and (c) 0.02% (w/v) benzalkonium chloride and 0.10% (w/v) sodium edetate.

After dissolving the ingredients in water, the final solution was heated in an autoclave at 121°C for 15 min.

2.3. Quantitative analysis

The quantitative analysis was carried out by high-performance liquid chromatography (HPLC), using a component system consisting of a Milton Roy ConstaMetric 3000 solvent delivery system, a Rheodyne 7125 injector, a LiChrosorb® RP-18 5 μm (150 × 4,0 mm) column and a Spectra-Physic SP8450 UV/VIS detector operated at 245 nm. The mobile phase consisted of acetonitrile, tetrahydrofuran and water (35:1:64). The retention time was 2.8 min at 1.50 ml/min flow rate.

2.4. Stability testing

Hydrocortisone solutions (0.30% w/v) in 2-hy-

droxypropyl-β-cyclodextrin 4.5% (w/v) were prepared at pH 4.5, 6.0 and 7.0. The stability of the solutions was tested at 2.5, 20 and 53°C. Oxygen-free solutions were prepared by purging with N_2 for 5 min. Three samples were taken at the beginning of the experiment and then at a weekly interval over a period of 8 weeks. The samples were kept frozen until they were assayed by HPLC. The effect of heating the solutions in an autoclave at 121°C for 15 min on hydrocortisone stability was also examined.

2.5. Test for preservative efficacy

2.5.1. Tests with microorganisms

Tests for preservative efficacy of the hydrocortisone solutions were carried out using methods similar to those described in the British Pharmacopoeia (1993). Standard test organisms *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 28516) and *Aspergillus niger* (laboratory isolate) were obtained from the clinical microbiology laboratory of the National University Hospital of Iceland. A clinical isolate of *Streptococcus mitis* was obtained from the microbiology laboratory of the Faculty of Odontology of the University of Iceland.

Overnight cultures of test organisms in Brain Heart Infusion Broth (Difco) were diluted in broth as required to yield approximately 10^7–10^8 colony forming units per millilitre (CFU/ml). *Aspergillus niger* was not diluted but used directly from a sporulating broth culture. Test strains were used to inoculate 20-ml samples of the hydrocortisone mouthwash to yield 10^5–10^6 CFU/ml final concentration so that the bacteriological growth medium was no more than 1% of the final volume of mouthwash (BP 1993). Aliquots of each inoculum were used to determine, by standard methods, the number of microorganisms (CFU/ml) added to each sample of mouthwash. All samples of mouthwash were stored at room temperature (21–22°C) protected from light. After 48 h, 7 and 28 days, samples (1 ml) were removed from the mouthwash preparations for determination of the viable count of each test organism. In order to remove the inhibitory effect of the preservatives in the mouthwash, the sample was centrifuged (MSE bench centrifuge 2500 r.p.m. for 20 min) and the clear supernatant discarded. The bacterial pellet was washed twice in PBS and finally re-suspended in PBS to exactly 1 ml. Viable counts of test organisms were then determined, again using standard methods. The remaining sample was filtered using a syringe filter with a removable filter disk (Whatman 0.45 μm pore cellulose nitrate membrane filter). This filter was then placed on a blood agar plate and incubated as a further check for the presence of viable organisms. For the mouthwash sample inoculated with *A. niger*, 1 ml of the sample was removed at 48 h, 7 and 28 days, washed and used to inoculate blood agar plates which were then incubated to ascertain the presence of viable organisms.

2.5.2. Tests with saliva

To a 20 ml sample of mouthwash was added 25 μl of fresh, stimulated human saliva, once per day for 1 week. The sample was shaken and stored in the dark at 21–22°C. Survival of organisms was tested by taking a 1-ml sample of the mouthwash, diluting it and inoculating blood-agar plates. Samples were taken just after the addition of the seventh sample (day 0), after 2, 7 and 14 days.

2.6. Preliminary clinical evaluation

Consecutive patients, referred to either a specialist Oral Medicine clinic or to the Oral Medicine clinic of the Faculty of Odontology, University of Iceland, with lesions suitable for treatment with topical steroids were asked to participate in the study. Patients were examined clinically by one of the authors (WPH) and all gave their informed consent after examination, investigation and the establishment of a clinical diagnosis. Patients with a concurrent viral or yeast infection were excluded from the trial. A total of 50 patients were entered into the study.

Subjects were asked to rinse their mouths with 10 ml of the hydrocortisone mouthwash three times daily. Rinsing was to be for 1–2 min after which the mouthwash was expectorated. Patients were asked to rinse for 2 weeks initially after

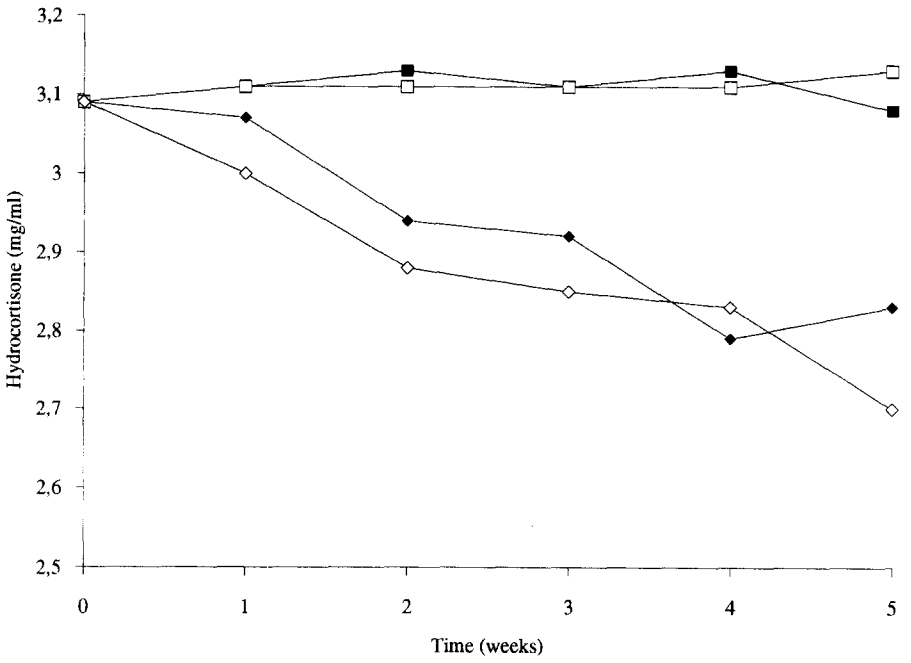

Fig. 1. Effect of temperature and the presence of nitrogen on the degradation of hydrocortisone. –■– 2.5°C (+ N_2); –□– 20°C (+ N_2); –◆– 53°C (+ N_2); –◇– 53°C (– N_2).

which they were re-examined. The trial received approval of the relevant University ethical authorities and the Icelandic Committee on Drugs.

3. Results and discussion

3.1. Formulation of solutions

At room temperature, the solubility of hydrocortisone in water is about 0.3 mg/ml and the stability of the 2-hydroxypropyl-β-cyclodextrin (1:1) complex has been estimated to be 1000 M^{-1} (Loftsson et al., 1991b). Thus, about 97% of the hydrocortisone is bound to cyclodextrin in the mouthwash solution. Only 3% (or about 0.01 mg/ml) is in the free form. The large hydrophilic cyclodextrin molecules do not permeate lipophilic biological membranes and, thus, cyclodextrin complexation could reduce the bioavailability of drugs. However, heating the solution to 121°C for 15 min promotes formation of drug-cyclodextrin-polymer co-complexes which have been shown to enhance drug delivery through biological membranes, such as hairless mouse skin (Sigurdardóttir and Loftsson, 1995). Through complexation, the hydrophilic 2-hydroxypropyl-β-cyclodextrin increases the aqueous solubility of hydrocortisone without changing the lipophilicity of the hydrocortisone molecule (i.e. reducing its ability to permeate lipophilic biological membranes). Enhanced bioavailability is obtained by making the hydrocortisone molecule more available to the oral mucosa (Loftsson and Bodor, 1995).

The results from the stability testing at different temperatures are shown in Fig. 1. No degradation was observed at 2.5 and 20°C but some decline in the hydrocortisone concentration was observed at 53°C. Elimination of oxygen from the reaction medium did not appear to have any effect on the degradation rate. The shelf-life of the hydrocortisone solution was estimated to be between 5 and 6 weeks at 53°C.

The effect of autoclaving the solutions at different pH values can be seen in Fig. 2. The results show that maximum stability is obtained at pH 4.5 and stability is not improved by purging the solution with nitrogen before heating.

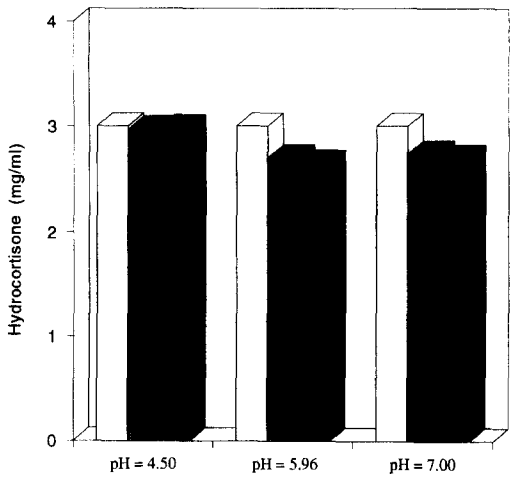

Fig. 2. Effect of pH and the presence of nitrogen on hydrocortisone stability when heated at 121°C for 15 min. □ before autoclaving; ▨ after autoclaving (+ N_2); ■ after autoclaving (− N_2).

It has been shown previously that 2-hydroxypropyl-β-cyclodextrin interacts with several commonly-used preservatives (Loftsson et al., 1992). The preservative molecules can displace drug molecules from the cyclodextrin cavity reducing the solubilizing and stabilizing effect of the cyclodextrin and the complexation of the preservatives reduces, or even abolishes, their antimicrobial activity. Hydrocortisone solutions were prepared using methyl parahydroxybenzoate and a combination of benzalkonium chloride and sodium edetate in two different concentrations. Antimicrobial preservative efficacy testing was carried out using methods similar to those described in the British Pharmacopoeia 1993; this test involves contaminating the solution to be tested with certain microbes and then evaluating the preservative effectiveness. In addition to the organisms specified for the use in the testing procedure by the BP 1993, i.e. *Aspergillus niger*, *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the solution was also tested for *Escherichia coli* and *Streptococcus mitis*. Results for a hydrocortisone solution containing 0.02% benzalkonium chloride and 0.1% sodium edetate are shown in Table 1 and they demonstrate that all organisms declined or died in the mouthwash by 48 h. The fall in log count for *S. aureus* was just less than 3 at 48 h but the count declined to zero by 7 days. The inoculum was at the high point of the levels advised by BP 1993. At the 7th day, only *C. albicans* was still viable but counts of this organism did not rise again even by the 28th day. The mouthwash sample contaminated with saliva daily for 1 week yielded 10^4 cfu/ml on blood agar at day 0 but no organisms were recovered thereafter at days 2, 7 or 14.

This mouthwash clearly demonstrates preservative efficacy assessed using methods similar to, but somewhat more strict than BP 1993. BP 1993 requires a log reduction of 3 in 14 days for oral preparations but this level should be achieved in 48 h for topical preparations.

3.2. Preliminary clinical evaluation

The mouthwash was well tolerated by patients with no ill effects or side effects being reported. No case of superinfection was observed (Holbrook et al., 1994). Clinical benefit of the mouth wash was assessed on a subjective five-point scale as shown in Table 2. Nine patients all with aphthous

Table 1
Survival of micro-organism in the hydrocortisone mouthwash containing 0.02% benzalkonium chloride and 0.1% sodium edetate

	0 h (cfu/ml)	48 h (cfu/ml)	7 days (cfu/ml)	28 days (cfu/ml)
Staphylococcus aureus	1.1×10^6	4.4×10^3	0	0
Pseudomonas aeruginosa	8.6×10^5	0	0	0
Streptococcus mitis	5.0×10^5	0	0	0
Escherichia coli	6.6×10^5	0	0	0
Candida albicans	3.7×10^6	2.4×10^5	2.4×10^4	2.4×10^4
Aspergillus niger	Growth	—	No growth	No growth

Table 2
Results of clinical evaluation of hydrocortisone mouthwash

Clinical results	No. of patients (%)	
Cured	9	(18)
Much better	15	(30)
Better	15	(30)
Same	11	(22)
Worse	0	(0)
Evidence of superinfection	0	(0)
Complaint of irritation	0	(0)

stomatitis were recorded as 'cured' as no recurrence of oral ulceration was noted in the 6 months following the use of hydrocortisone for 2 weeks. Thirty patients (60%) regarded their symptoms as reduced or even much reduced and this included the marked reduction of many erosions in patients with lichen planus. Eleven patients found no benefit from the mouthwash but no patient reported a worsening of symptoms. The clinical results are sufficiently promising to warrant a clinical trial. As no similar preparation is on the market and the only commercially-available steroid for oral use is Kenalog in Orabase®, a placebo-controlled trial seems to be the most promising way to test clinical efficacy, but the ethical considerations of such a trial are problematical.

4. Conclusion

Topical steroids remain the most useful medicaments for oral conditions such as erosive lichen planus or aphthous stomatitis. When mucosal ulceration is widespread, it becomes difficult to apply topical creams and so a mouthwash preparation offers considerable advantages. The trend in recent years has been to use ever more potent steroids such as clobetasol (Rödström et al., 1992) but the persistence of lesions and the frequency of recurrence can lead to complications after long-term use of potent steroid. The present mouthwash is stable and meets strict criteria for preservative efficacy. It has a clinical efficacy that compares well with much stronger preparations and should help to minimise the prevalence of harmful side effects of the treatment of these distressing conditions.

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