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Formulation development and in vivo evaluation of a novel bioadhesive lozenge containing a synergistic combination of antifungal agents

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Abstract

Two novel bioadhesive antifungal lozenges were developed. Both were two-layered with an upper modified-release drug-containing layer and a lower bioadhesive layer composed of drum-dried waxy maize starch and Carbopol 980 to facilitate application to the oral mucosa. The first type of lozenge contained miconazole nitrate as a spray-dried form containing acacia and Cremophor RH40 to increase the dissolution of the poorly soluble azole, plus flavourings. The second type also contained chlorhexidine acetate in the drug layer, as both drugs had been reported to act synergistically. In vivo release of drug(s) into saliva was assessed in a group of healthy volunteers, with the lozenges located in an upper posterior site in the oral cavity. Therapeutic levels were achieved for extended periods of time with both formulations. Intra-subject variation was greater than inter-subject variation. By examining salivary drug concentrations obtained when the second formulation was applied to an upper anterior location, the release of drugs was shown not to be significantly affected by the location within the oral cavity. In comparison to a proprietary oral gel formulation, the new bioadhesive lozenges produced much more uniform and effective salivary levels of miconazole nitrate over a prolonged period.

Keywords: Bioadhesive lozenges; Miconazole nitrate; Chlorhexidine acetate; Synergy; Salivary levels

1. Introduction

Miconazole nitrate is widely used in the treatment of oral candidosis, a fungal infection caused primarily by *Candida albicans*. Local treatment causes less systemic side-effects and increased concentration where required with reduced duration of treatment. Commercially it is available as Daktarin® oral gel (2% miconazole nitrate), a product whose salivary concentration of drug has been shown to fall below the minimum inhibitory con-

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centration for *C. albicans* within 30 min (Odds, 1981; Turner and Warnock, 1982; Bouckaert et al., 1992). Pedersen and Rassing (1990) found that solid dispersions of miconazole and PEG 6000 increased the release of the drug from chewing gum, whereas other carriers such as PVP, xylitol or urea were ineffective. Pedersen and Rassing (1991) used Panodan® 165 (an anionic solubilizing agent) to improve the release of miconazole from chewing gum. A stability problem with Panodan has been reported recently, necessitating its replacement with a more stable surfactant (Rassing, 1994). Pedersen (1993) showed also that inclusion complexes of clotrimazole with cyclodextrins improved drug solubility and helped mask its taste. Chlorhexidine gluconate 0.2% is used locally as a flavoured mouthwash (Corsodyl®).

Possible approaches to the formulation of bioadhesive extended-release lozenges have been reported by Collins and Deasy (1990), Bottenberg et al. (1991) and Ponchel (1994), multilayer devices being favoured. We have recently reported (Codd and Deasy, 1998) that miconazole nitrate and chlorhexidine acetate have synergistic antifungal activity against *C. albicans*. This paper describes the in vitro development and aspects of the in vivo assessment in humans of novel bioadhesive lozenges containing these antifungal agents.

2. Materials and methods

2.1. Materials

Acacia (Koch-Light), acetonitrile (HPLC grade), methanol (HPLC grade, Rathburn), benzaldehyde (Aldrich), bovine oral mucosa (Kepak), *Candida albicans* (Department of Microbiology, University of Dublin), Carbopol® 980 (C980, Goodrich), chlorhexidine acetate (ICI), Cremophor® RH40 (BASF), Daktarin oral gel (batch number 94G29/X292, Janssen), drum-dried waxy maize starch (DDWMS, Cerestar), econazole nitrate, miconazole nitrate, naproxen, triethylammonium phosphate (Sigma), *n*-heptane (HPLC), methanol, sodium hydroxide, sulphuric acid (Riedel-de Haën), nitrogen (oxygen-free, Irish Industrial Gases), Sabouraud's dextrose agar (Oxoid), sodium saccharin, vanillin (BDH), sodium sulphate (May & Baker), spray-dried orange flavour (Döhler), tetrahydrofuran (HPLC grade, Lab-Scan), triethylamine (Merck) and glass-distilled water were used. All reagents were GPR unless otherwise indicated.

2.2. Preparation of bioadhesive lozenges

Two lozenge formulations (A and B) were assessed in the oral cavity. The lower flat-faced bioadhesive layer of the 8.5 mm compacts compressed under 1 ton was composed of 20 mg of a physical mix of DDWMS 95%/C980 5%. This layer was optimized after a screen of potential polymers assessed for bioadhesive property using a Stable Micro Systems XT.RA dimension texture analyser with bovine mucosa from the soft palate and in vivo studies in the oral cavity of a panel of humans for duration of attachment and post-removal irritancy. The outer convex-faced layer was composed of 150 mg of either spray-dried miconazole nitrate/Cremophor RH40/acacia, 1:10:14 (powder A) and flavouring, or miconazole nitrate/chlorhexidine acetate/Cremophor RH40/acacia, 1:0.5:10:14 (powder B) and flavouring. Preliminary studies showed that Cremophor RH40, which is a solubilizing agent and because of its paste-like consistency was best incorporated by spray-drying with suitable carriers, was more effective than a range of weak acid pH adjusters or enteric polymers at increasing miconazole solubility. The content of both drugs was confirmed in the spray-dried powders by assay, and to the quantity containing 5 mg miconazole nitrate was added sufficient flavouring to 150 mg. The flavourings used to mask the unpleasant taste of the drugs were sodium saccharin, spray-dried orange, vanillin and benzaldehyde. The maximum height of the compacts was 2.5 mm. The final formulation of type A lozenges contained an upper layer composed of powder A 134 mg, spray-dried orange 13 mg, saccharin 0.7 mg, vanillin 2.3 mg, benzaldehyde 10 μl, and a lower layer composed of DDWMS 19 mg, C980 1 mg. The final formulation of type B lozenges contained an upper layer composed of powder B 130 mg, spray-dried orange 17 mg, saccharin 0.7 mg, vanillin 2.3

mg, benzaldehyde 10 μl, and a lower layer composed of DDWMS 19 mg, C980 1 mg.

2.3. In vivo study design

A panel of eight healthy subjects (three male, five female, aged 24–54 years) was used in a random cross-over study designed to determine salivary levels of active following application of new bioadhesive lozenges compared to the proprietary product Daktarin gel (Janssen). A written protocol was provided for participants, who applied each product following thorough cleaning of the teeth 30 min after breakfast. A standard meal was provided 305–335 min after product administration, and during the trial subjects were allowed to drink water from 120 min onward. No water was allowed 10 min before collection of saliva samples and care was taken to ensure that during this period the tongue did not contact the product to avoid producing abnormally high drug levels.

Each tablet was applied to the oral cavity by placing the flat bioadhesive surface against the gingiva (from which the excess saliva had been blotted) and applying slight pressure for 20 s. The exposed convex face of the tablet was moistened with the tongue to prevent the tablet sticking to the cheek. Tablet A was applied to the left upper attached gingiva in the region of the second molar. Tablet B was applied either to the same site (tablet B1) or to the left upper attached gingiva in the region of the canine (tablet B2).

Prior to an application, a blank sample of saliva was taken and further samples were subsequently taken at periodic intervals up to 9 h after application of a tablet and up to 6 h after application of the Daktarin gel (3 g spread around the mouth using the tongue and held for 60 s before swallowing). Approximately 2 ml of saliva was collected over a 2-min period (1 min before and 1 min after the stated time) and stored at −20°C prior to analysis. Subjects scored products in terms of comfort, taste and irritancy, for preference between products, and for the duration of lozenge presence in the oral cavity.

Miconazole nitrate levels in saliva from products were determined using a specially developed bioassay, employing measurement of zones of inhibition caused by absorbent discs impregnated with test media and standards on the surface of Sabouraud's dextrose agar seeded with 3×10^6 cells/ml of a laboratory strain of *C. albicans*, prior to incubation at 37°C for 18 h (Codd, 1996). The assay was validated for day-to-day precision (4.1% and 5.7% S.D.) and linearity (0–100 μg/ml, $r^2 = 0.998$ and 0–200 μg/ml, $r^2 = 0.999$) for bioadhesive lozenges and Daktarin gel, respectively, by examination of the equations of the lines of best fit of calibration curves determined on five consecutive days with regard to slope and r^2. Intra-day precision following repeat assay of 10, 100 and 200 μg/ml samples gave S.D. values of 4.7%, 5.8% and 5.7%, respectively. The limit of detection was 0.5 μg/ml.

Miconazole nitrate concentration in salivary samples was determined also by HPLC using an assay modified from Di Pietra et al. (1992) and Codd (1996). The assay was validated for inter-day precision (2.6% S.D.), linearity (0–60 μg/ml, $r^2 = 0.995$), intra-day precision at 40 μg/ml (1.2% S.D.), recovery (at 8 and 40 μg/ml, $85.0 \pm 2.6\%$ and $89.6 \pm 1.8\%$ S.D., respectively), limit of detection (0.75 μg/ml) and specificity for miconazole nitrate (no interfering peaks observed). Daktarin gel could only be assayed by the disc diffusion bioassay because of interference at miconazole nitrate's retention time by components in the gel.

Chlorhexidine acetate salivary levels from tablets B1 and B2 were determined by HPLC using an assay modified from Gadde et al. (1991) and Codd (1996). The assay was validated for inter-day precision (1.7% S.D.), linearity (0–30 μg/ml, $r^2 = 0.999$, intra-day precision at 25 μg/ml (0.4% S.D.), recovery (at 5 μg/ml, $97.6 \pm 2.6\%$ S.D.), limit of detection (0.5 μg/ml) and specificity for chlorhexidine acetate (no interfering peaks observed).

3. Results and discussion

3.1. Formulation of bioadhesive lozenges

It was considered that a bioadhesive lozenge containing 5–10 mg of miconazole nitrate would produce acceptable salivary levels of miconazole

Table 1
Pharmacokinetic parameters calculated from the release profiles using the disc diffusion bioassay after application of type A bioadhesive lozenges

Subject	AUC (μg/ml.min)	$T^{>MIC}$ (min)	C_{max} (μg/ml)	t_{max} (min)
1	13.0	300	120.5	120
2	6.4	150	93.5	75
3	4.9	90	74.1	60
4	5.0	240	47.0	75
5	10.4	270	58.4	150
6	8.2	240	74.9	75
7	4.8	195	46.9	90
8	10.0	180	108.6	75

AUC is the area under the curve calculated using the trapezoidal method.
$T^{>MIC}$ is the time that miconazole nitrate levels exceed a MIC value of 5 μg/ml.
C_{max} is the maximum salivary concentration of miconazole nitrate.
t_{max} is the time to reach C_{max}.

nitrate over a prolonged period of time (Bouckaert et al., 1992), lower drug loading favouring the production of a less bulky product. Consequently in this study it was decided to develop lozenges (type A) containing an upper layer with 5 mg of the drug provided by powder A having enhanced drug dissolution. A second lozenge (type B) combined 5 mg miconazole nitrate with 2.5 mg chlorhexidine acetate (powder B) as maximum synergy had been established previously against *Candida* for this ratio of actives (Codd and Deasy, 1998). Both lozenge types contained flavouring agents in the upper layer. Sodium saccharin, orange oil, cocoa flavour, vanillin and benzaldehyde are present in Daktarin gel in order to mask the unpleasant metallic taste which miconazole possesses. Chlorhexidine acetate is also an unpleasant tasting substance, further necessitating the inclusion of flavouring agents. Preliminary evaluation of a range of flavours for the proposed lozenges in two subjects gave rise to the quantities specified.

3.2. In vivo assessment in one location of bioadhesive lozenges containing miconazole nitrate

Salivary concentrations of miconazole nitrate from type A bioadhesive lozenges located on the left upper gingiva in the region of the second molar in eight healthy subjects were estimated by disc diffusion bioassay and HPLC. Good agreement was seen between the two methods. For all of the subjects, the disc diffusion bioassay tended to be more sensitive than the HPLC method, detecting miconazole nitrate for longer periods. When the release profiles obtained by either assay method were compared at each time point using a two-sample t-test, the difference was not statistically significant, confirming the bioassay to be reliable alternative to HPLC.

Inspection of individual release profiles, showed that three of the subjects had an initial miconazole peak at the first sampling point (15 min), which then decreased to be followed by a subsequent increase in drug concentration that declined once more. This burst effect observed may have been due to poorly embedded drug at the surface of compacts as a consequence of fragmentation of spray-dried particles on compression, resulting in a reservoir of drug available for immediate release into the saliva. It may also have been related to the method of application of the bioadhesive lozenges, whereby the exposed convex surface was moistened with the tongue following insertion to prevent sticking to the cheek, as excessive moistening may have resulted in high initial salivary concentrations of drug. Two of the subjects showed a second small peak at the 240-min sampling point, which was presumably related to the final break-up of the lozenge in the oral cavity, releasing a burst of miconazole nitrate.

Table 2
Subjective assessment of type A lozenges in eight subjects

Comfort	*n*	Taste	*n*	Irritancy	*n*
Very comfortable	1	Very pleasant	0	None	4
Comfortable	6	Pleasant	2	Slight	3
Slightly uncomfortable	1	Slightly unpleasant	3	Moderate	1
Moderately uncomfortable	0	Moderately unpleasant	1	Severe	0
Very uncomfortable	0	Very unpleasant	2		

n is number of subjects.

Some pharmacokinetic parameters calculated from the release profiles are shown in Table 1. Considerable inter-subject variability was observed in all parameters measured. The mean AUC was 7.8 ± 3.1 μg/ml.min ($\pm$ S.D.). Effective extended release of miconazole nitrate was achieved after 15 min, with a mean $T^{>\mathrm{MIC}}$ of almost 3.5 h (208 ± 68 min), while the time taken to reach the C_{max} ($77.9 + 27.6$ μg/ml) varied from 60 to 150 min. The variability observed may have been due to factors such as individual facial and tongue movements, salivary flow and beverage intake. Overall, the subjects reported the lozenges to be quite comfortable, with little or no irritancy (Table 2). Taste perception varied from pleasant to severely unpleasant. On average, the subjects felt that the lozenges lasted nearly 3 h in the mouth (161.3 ± 45.2 min).

Intra-subject variability was considered in a single subject, who applied type A lozenges on five separate occasions and the results obtained were compared to those obtained from the other seven subjects as shown in Figs. 1 and 2. The intra-subject results were compared with the inter-subject results over the range of pharmacokinetic parameters, as shown in Table 3. As expected, it was observed that the release of miconazole nitrate was less variable, as denoted by lower S.D., when the lozenges were applied on a number of occasions to a single subject as compared to one occasion in several subjects. The mean C_{max} was slightly lower for the intra-subject variability study as compared to the inter-subject study, although not to a significant level ($p > 0.05$, one-way ANOVA). The AUC was higher for the single subject as a consequence of a more prolonged mean $T^{>\mathrm{MIC}}$ ($p > 0.05$) and a later t_{max}. The t_{max} value was the only parameter that had a significant difference between treatments ($p < 0.05$). However, it must be remembered that this value is affected by the sampling time points, which were selected arbitrarily and therefore may not show the actual time to reach maximum salivary concentration.

3.3. In vivo assessment in two locations of bioadhesive lozenges containing miconazole nitrate and chlorhexidine acetate

Type B1 lozenges were located in the posterior region of the oral cavity, applied to the upper left attached gingiva in the region of the second molar. When salivary levels of miconazole nitrate were determined by disc diffusion bioassay and HPLC, good agreement was again evident between the two methods of analysis. A later secondary peak in miconazole nitrate salivary concentration was obtained for all subjects, reflecting final erosion and break-up of the lozenge. Fig. 3 shows the ratio of miconazole nitrate to chlorhexidine acetate in saliva at each sampling time for each of the eight subjects. Almost all of the values fell within the range of miconazole nitrate/chlorhexidine acetate from 6:1 to 0.67:1, which from previous studies (Codd and Deasy, 1998) should exhibit good synergy between the drugs against *C. albicans*. The rate of release of the insoluble miconazole nitrate was much faster than that of chlorhexidine acetate at most of the sampling times, despite chlorhexidine acetate being the more soluble compound. It may be that the highly surface active compound chlorhexidine acetate was bound to excipients in the lozenge such as acacia or DDWMS, or to

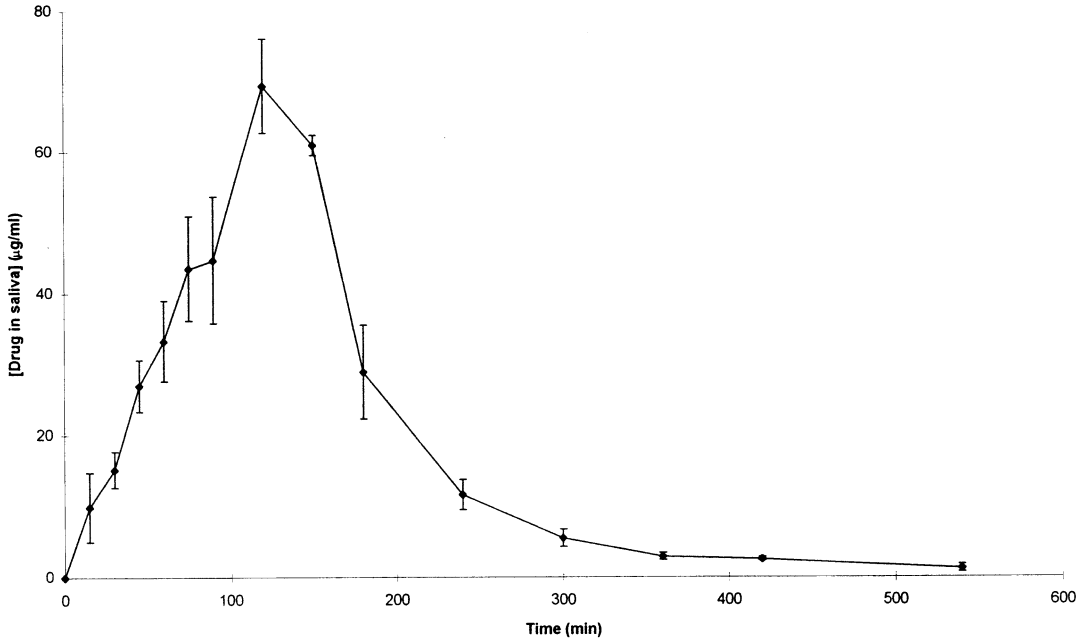

Fig. 1. Mean in vivo release of miconazole nitrate from type A lozenges applied in one subject on five occasions, analysed by disc diffusion bioasssay. Error bars represent ± 1 S.E.

bacteria, salivary proteins or plaque components in the oral cavity as observed by Bonesvoll et al. (1974), forming a temporary reservoir within the mouth that was unavailable for HPLC analysis. As time progressed, the release ratio decreased, probably due to depletion of miconazole nitrate from the tablet matrix. The greatest agreement between subjects for ratio of drugs release was seen at the sampling times 60, 75 and 90 min, which had an average release ratios of 3.1, 3.0 and 2.8, respectively.

The healthy volunteers reported the lozenges to be comfortable to wear, with a slightly unpleasant taste and some local irritation. Obviously such minor distaste and irritation would be more than balanced by the relief of symptoms in patients successfully treated by the product for conditions such as oral candidosis (oral thrush). The most common form of this disease is the acute pseudomembranous-type, were semi-adherent white patches appear as discrete or merging painful lesions on the surface of the buccal mucosa, throat, tongue and gum linings.

Type B2 lozenges were located in the anterior region of the oral cavity, applied to the upper left attached gingiva in the region of the canine tooth. When salivary levels of miconazole nitrate were determined, good agreement was observed again between both assay methods. When the ratio of miconazole nitrate to chlorhexidine acetate salivary concentrations was plotted against sample time (Fig. 4), the rate of release of miconazole nitrate was again faster than that of chlorhexidine acetate, probably due to binding of the latter drug in the oral cavity. These ratios were significantly higher for type B2 compared to type B1 lozenges at the sampling times 60, 75, 90 and 120 min ($p < 0.05$, two-sample t-test). Type B1 lozenges initially gave very high release ratios that decreased with time. Type B2 lozenges in comparison maintained their high release ratio for a longer period of time. This is probably due to their different locations in the oral cavity as in the posterior position (B1) salivary flow is greater than in the anterior position (B2). Therefore, miconazole nitrate was probably depleted faster from B1 than B2 lozenges, leading to a decrease

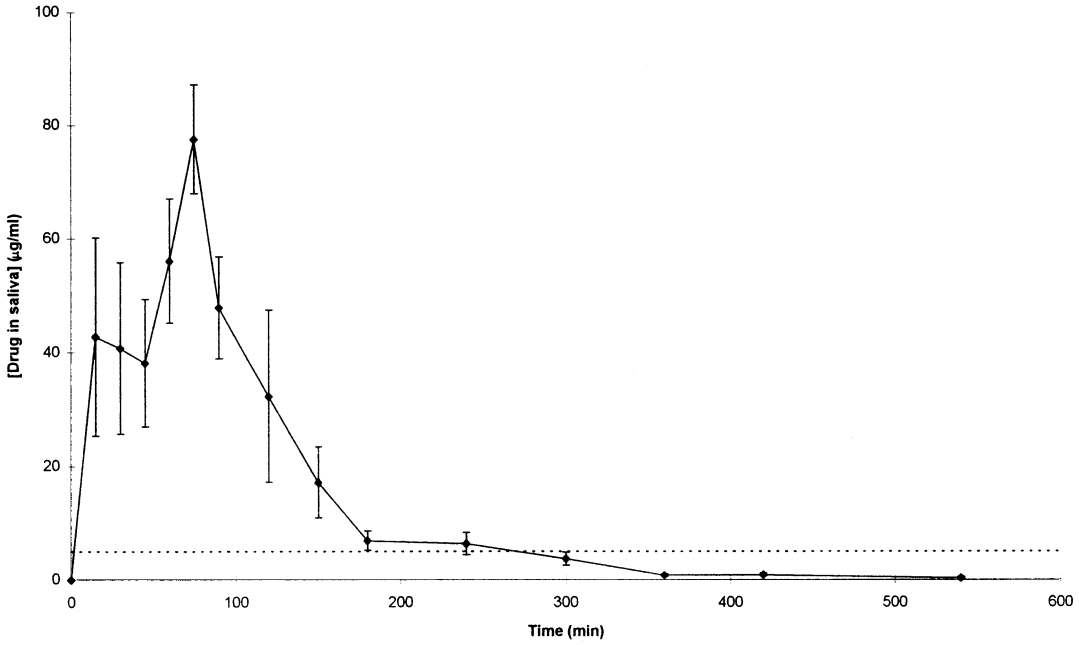

Fig. 2. Mean in vivo release of miconazole nitrate from type A lozenges applied in seven subjects on one occasion, analysed by disc diffusion bioasssay. Error bars represent ± 1 S.E.

in the drug release ratio for B1 lozenges with time. B1 lozenges released the drugs in a ratio closer to the preferred 2:1 miconazole nitrate/chlorhexidine acetate for maximum synergy.

Subjectively the type B2 lozenges were judged to be quite comfortable at the anterior location, with a slightly unpleasant taste and some minor local irritation reported in five of the subjects.

Figs. 5 and 6 show the mean salivary levels of both drugs with time for both locations. Because of substantial variation observed between subjects the plotted error bars are quite large and for clarity of presentation are not shown for the disc diffusion bioassay results. Other in vivo studies with sustained release tablets have reported this problem also (Tucker et al., 1989; Bottenberg et al., 1991; Bouckaert et al., 1992). Differences in release patterns are probably due to variable erosion of the lozenges due to differences in saliva flow and soft tissue movement as a result of speech and swallowing. Pharmacokinetic parameters were calculated from the data as shown in Table 4, where the MIC of chlorhexidine acetate was taken as 2.5 μg/ml (Codd and Deasy, 1998). When the lozenge was located in an anterior position, the release of miconazole nitrate was more sustained, reaching a higher salivary level and was slightly less variable than when in the posterior position in the oral cavity. However, because of the high inter-subject variability, these differences were not statistically significant when the mean results were considered ($p > 0.05$, one-

Table 3
Comparison of inter- and intra-subject pharmacokinetic parameters (mean $\pm$ S.D.)

Parameter	Inter-subject ($n = 7$)	Intra-subject ($n = 5$)
AUC (μg/ml.min)	7.5 ± 3.1	9.7 ± 1.2
%S.D.	41.3	12.4
$T^{>MIC}$ (min)	199 ± 69	243 ± 27
%S.D.	34.7	11.1
C_{max} (μg/ml)	80.8 ± 28.5	71.1 ± 12.9
%S.D.	35.3	18.1
t_{max} (min)	81 ± 19	132 ± 16
%S.D.	23.5	12.1

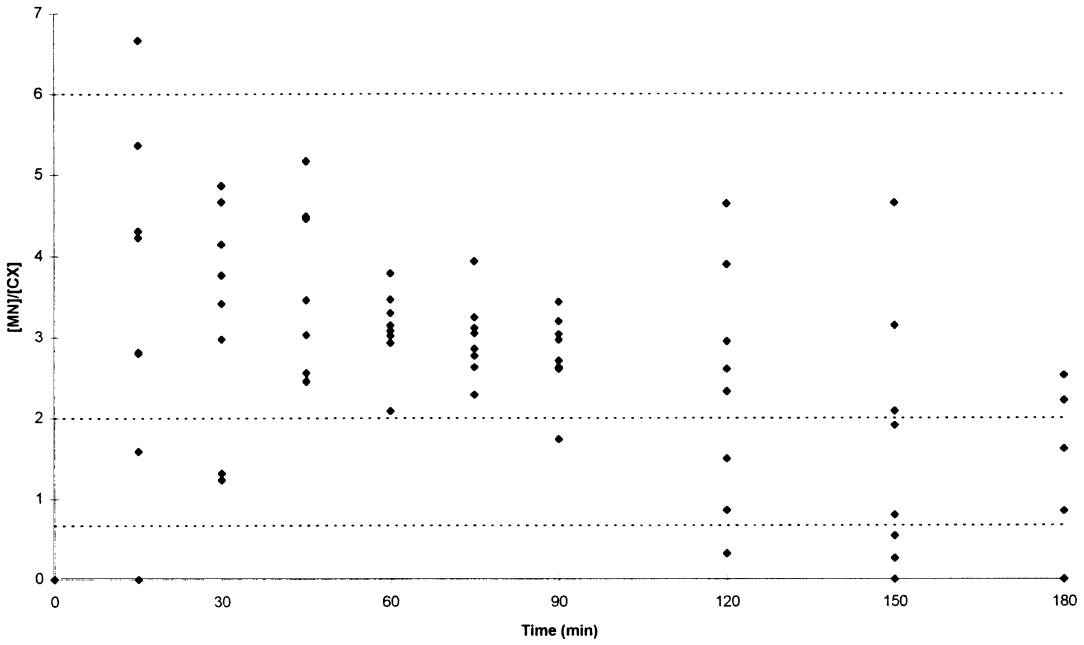

Fig. 3. Relationship between miconazole nitrate/chlorhexidine acetate salivary concentrations for type B1 lozenges in eight healthy subjects. The dotted lines represent 6:1, 2:1 and 0.67:1.

way ANOVA). The more sustained release effect in the anterior position, related to reduced salivary flow at this site, was confirmed by subjective assessment by the eight subjects who reported that the compacts lasted 207 ± 67 min (mean $\pm$ S.D.) when applied to the anterior region and only 167 ± 64 min when applied to the posterior region. Finally, there was no significant difference in chlorhexidine acetate release when the two locations were compared ($p > 0.05$, one-way ANOVA).

When the release of miconazole nitrate from type A and type B1 lozenges applied at the same location was compared, the mean release for the former type was more sustained ($T^{>\mathrm{MIC}}$ and $t_{\max}$ greater), reached a higher salivary level (AUC and $C_{\max}$ greater) and was slightly less variable (%S.D. AUC, $C_{\max}$ and $t_{\max}$ lower). However, because of high inter-subject variability, these differences were not statistically significant ($p > 0.05$). The inclusion of soluble chlorhexidine acetate in type B1 lozenges may have affected properties of the compact such as level of cohesiveness, porosity and wettability, and could have acted as a channelling agent facilitating diffusion of saliva into the compact to aid its disruption, with greater release and with quicker clearance of poorly soluble miconazole nitrate.

The salivary levels of miconazole nitrate were assessed using the disc diffusion bioassay after treating the eight subjects with 3 g of Daktarin oral gel (60 mg miconazole nitrate), as shown in Fig. 7. The recommended therapeutic dose of this gel is half of a 5-ml spoonful (equivalent to 62 mg miconazole nitrate) four times a day. All the subjects showed a peak concentration at 5 min which on average rapidly declines below the MIC after 40 min. Subjective assessment of Daktarin oral gel was favourable, although three of the subjects did not like the sensation of the gel in the mouth, complaining of nausea. Overall the product was considered to be pleasant tasting with no associated irritation.

The release of miconazole nitrate from Daktarin gel was compared with that from type A lozenges. The AUC, $T^{>\mathrm{MIC}}$ and $t_{\max}$ were signifi-

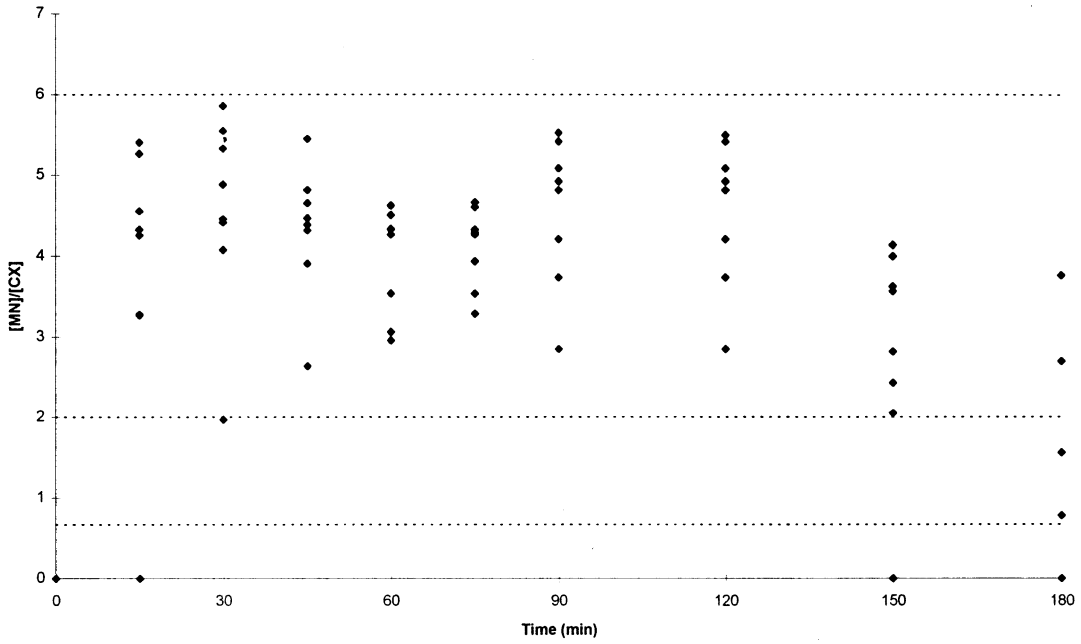

Fig. 4. Relationship between miconazole nitrate/chlorhexidine acetate salivary concentrations for type B2 lozenges in eight healthy subjects. The dotted lines represent 6:1, 2:1 and 0.67:1.

cantly higher for the bioadhesive lozenge than from the proprietary gel ($p < 0.05$, two-sample t-test). The mean AUC for the bioadhesive lozenge was almost four times greater than the mean AUC for the gel, even though the latter product contained 12 times as much drug. The time during which the salivary concentration exceeded the MIC was only 41 ± 35 min (mean $\pm$ S.D.) for the gel, compared to 208 ± 68 min for the bioadhesive lozenge. Similar differences were observed when type B1 and B2 lozenges were compared to the gel. Again the AUC, $T^{>\mathrm{MIC}}$ and t_{max} were significantly higher for the bioadhesive lozenges than for Daktarin gel ($p < 0.05$, two-sample t-test). The mean AUC for type 1 and 2 lozenges were 2.7 and 3.1 greater than the gel, respectively, while the duration that the miconazole concentration was above the MIC was 158 ± 45 and 182 ± 76 min, respectively. Only one subject preferred the bioadhesive lozenges to the oral gel. Three preferred the gel and four subjects had no preference.

3.4. Conclusions

It was decided to examine two different sites of application for type B lozenges. The first was an upper posterior location on the attached gingiva in the region of the second molar, where the lozenge should be unobtrusive and adequate salivary flow should ensure good distribution of the drug throughout the oral cavity. The second was an upper anterior location on the attached gingiva in the region of the canine, where the amount of mucosal movement should be small, salivary flow poor and clearance of the drug slow (Lecomte and Dawes, 1987; Bouckaert et al., 1993; Rathbone et al., 1994; Weatherell et al., 1994). In practice, location of the lozenge in the anterior position gave slightly more sustained and higher levels of miconazole nitrate than the posterior position. However, when the flow pattern of saliva in the oral cavity is considered, locating the lozenge in a posterior site may result in more effective distribution of antifungal throughout the

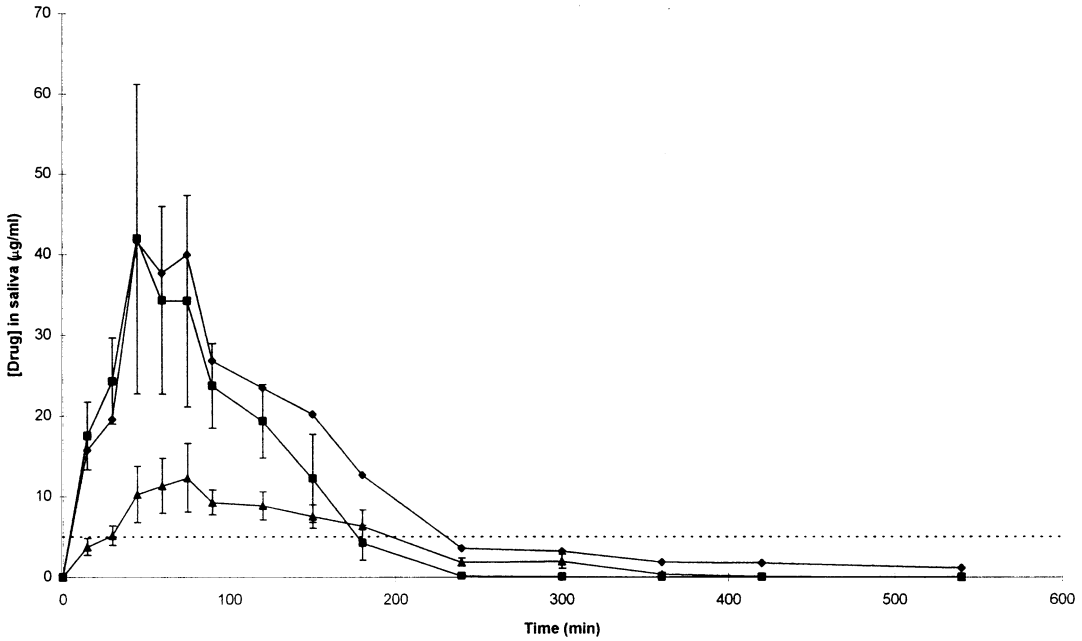

Fig. 5. Mean in vivo release of drug in eight subjects from type B1 lozenges: miconazole nitrate analysed by disc diffusion bioassay (– ◆ –) and HPLC (– ■ –); chlorhexidine acetate analysed by HPLC (– ▲ –). Error bars represent ±1 S.E. The dotted line represents the MIC of miconazole nitrate (5 μg/ml).

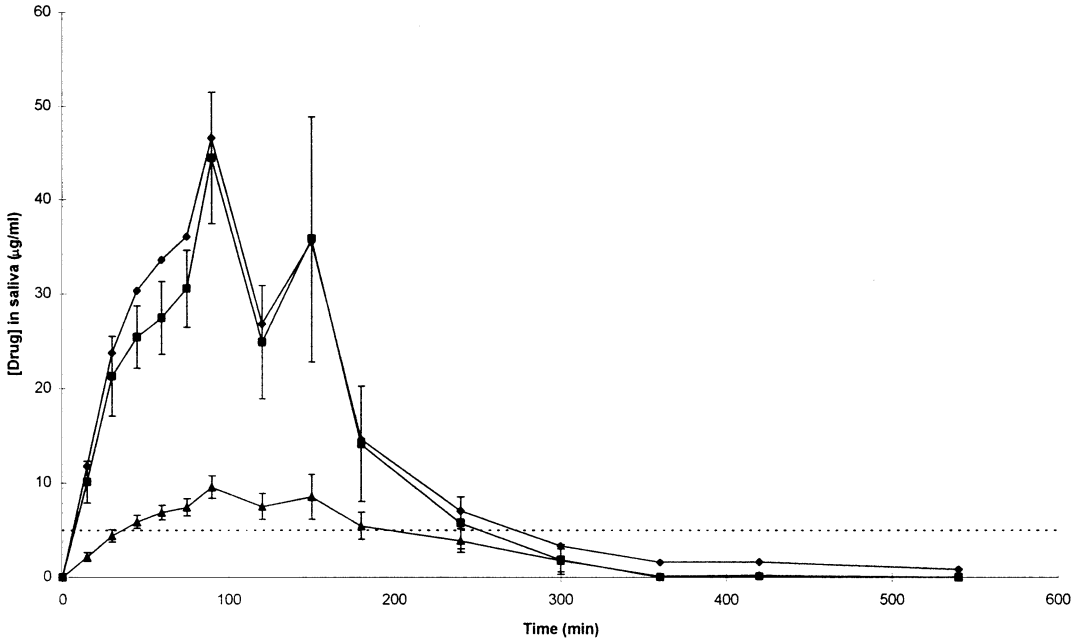

Fig. 6. Mean in vivo release of drug in eight subjects from type B2 lozenges: miconazole nitrate analysed by disc diffusion bioassay (– ◆ –) and HPLC (– ■ –); chlorhexidine acetate analysed by HPLC (– ▲ –). Error bars represent ±1 S.E. The dotted line represents the MIC of miconazole nitrate (5 μg/ml).

Table 4
Pharmacokinetic parameters (mean ± S.D.) for miconazole nitrate and chlorhexidine acetate salivay levels resulting from application of lozenges to two locations in the oral cavity

Parameter	Miconazole nitrate		Chlorhexidine acetate	
	Location B1	Location B2	Location B1	Location B2
AUC (μg/ml.min)	5.6 ± 2.7	6.5 ± 2.3	1.9 ± 1.1	1.6 ± 0.7
%S.D.	48.2	35.4	57.9	43.8
$T^{>MIC}$ (min)	158 ± 45	182 ± 76	188 ± 71.3	174 ± 65.6
%S.D.	28.5	41.7	37.8	37.7
C_{max} (μg/ml)	52.2 ± 50	58.5 ± 21.3	14.8 ± 10.6	11.8 ± 4.4
%S.D.	95.8	36.4	71.6	37.2
t_{max} (min)	77 ± 41	96 ± 37	103 ± 38	98 ± 34
%S.D.	53.2	38.5	36.9	34.7

oral cavity, leading to a therapeutically superior product in diseased individuals.

When compared to Daktarin gel, type B lozenges in either location gave superior release profiles of miconazole nitrate with regards to both salivary concentrations achieved and the duration these levels were maintained. The prolonged 2:1 release pattern for miconazole nitrate/chlorhexidine acetate previously observed during in vitro studies was not observed in vivo, presumably due to differences in hydrodynamics between the dissolution set-up (1 l phosphate buffer pH 6.85) and the oral cavity (1 l per day, pH 6–7.4) whose continuous flow would promote better sink conditions. Instead the ratio varied from about 1:1 to 7:1, which should still result in synergistic interaction leading to superior therapeutic response. The increased miconazole nitrate release and the decreased availability of chlorhexidine acetate due to oral binding would explain the higher release ratios reported in vivo as compared to in vitro.

When using type B lozenges, they probably should be applied to the posterior upper gingiva region with a frequency of one, four times a day.

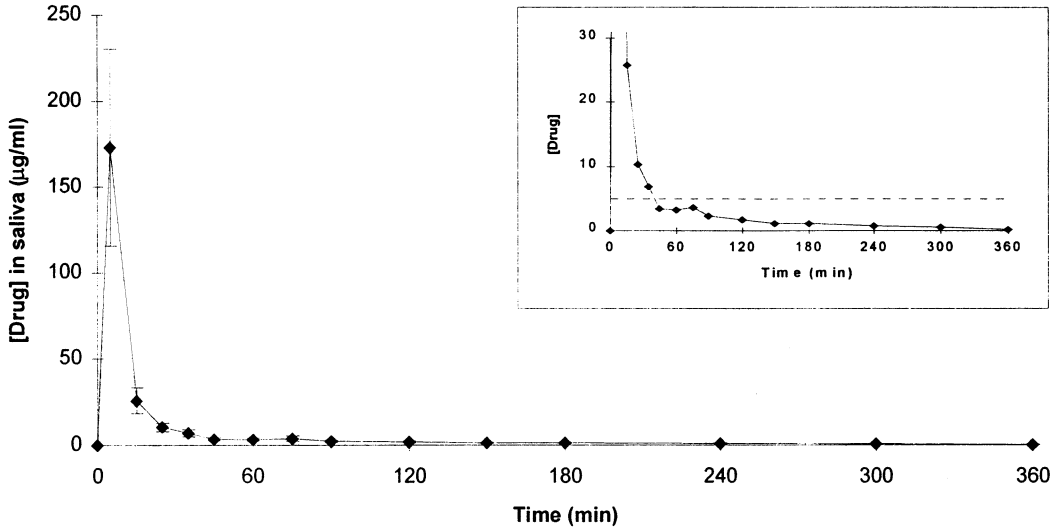

Fig. 7. Mean release of miconazole nitrate from Daktarin oral gel in eight subjects. Error bars represent ± 1 S.E. The dotted line on the inlayed plot represents the MIC of miconazole nitrate (5 μg/ml).

No beverages or food should be permitted for 2 h, which in practice would indicate that the product should be applied between meals. The flavour of the lozenge needs further improvement. The location of the device should probably be varied to reduce any slight local irritation.

The bioadhesive lozenges await comparative therapeutic evaluation against existing proprietary products in a group of patients with diseased states amenable to treatment with the combination of antifungal agents contained therein.

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