In Vitro/in Vivo Correlation of the Bioadhesive Properties of a Buccal Bioadhesive Miconazole Slow-Release Tablet

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Received July 13, 1992; accepted December 4, 1992

An *in vitro–in vivo* correlation study was performed on the bioadhesive properties of three buccal formulations based on modified starch (drum-dried waxy maize)/polyacrylic acid mixtures. Mixtures containing 10 mg miconazole nitrate, characterized by a different *in vitro* detachment force and work of adhesion, were evaluated for their bioadhesive properties in human volunteers. The results obtained showed that no significant difference could be seen among the formulations *in vivo*. The *in vitro* method showed no significant influence of miconazole nitrate on the bioadhesion properties of the polymers, while the *in vivo* adhesion time of the pure polymer mixtures was significantly higher than for the polymers containing miconazole. The results from the *in vitro* method thus did not correlate well with the *in vivo* data. The *in vitro* method provided information only on the initial bioadhesion and no correlation could be made with the residence time of the tablet in the oral cavity.

KEY WORDS: miconazole; buccal bioadhesive; slow release; *in vitro/in vivo* correlation.

INTRODUCTION

Bioadhesive controlled-release systems have been thoroughly studied recently (1–3). To determine the bioadhesive potential of different polymers, several techniques were reported (4–6), mostly involving the measurement of adhesive strength and using *ex vivo* tests. Some of these tests are used to classify polymers according to their adhesive properties. A technique based on a tensile testing apparatus was developed by Ponchel *et al.* (7), determining the detachment force and the work of adhesion when tablet and tissue are pulled apart. Using this approach we determined the bioadhesive characteristics of tablets consisting of thermally modified starch and polyacrylic acid. The results suggested that the combination of thermally modified starch with 5% polyacrylic acid is promising for the development of a buccal bioadhesive slow-release tablet (8). In order to assess the significance of detachment force and work of adhesion for the *in vitro* prediction of the mucoadhesive properties of formulations in the human buccal cavity, an *in vitro/in vivo* correlation study was performed.

Three formulations, based on a mixture of thermally modified starch (drum-dried waxy maize; DDWM) and 5% of three types of polyacrylic acid, with known but different *in vitro* detachment force and work of adhesion, were evaluated *in vivo* in human volunteers. In a first study, formulations containing miconazole nitrate were evaluated. The *in vivo* adhesion time of the tablet and the release profile of miconazole nitrate were compared according to their previously determined *in vitro* bioadhesion characteristics. In a second study, the *in vivo* adhesion times of the pure polymer formulations were compared to their *in vitro* bioadhesion properties and to the adhesion time of the drug-loaded polymers, in order to evaluate the influence of the drug in the polymer formulation.

MATERIALS AND METHODS

Drug Product

The bioadhesive tablets[4] were prepared using a polymer mixture of 5% polyacrylic acid (BF Goodrich Co, Cleveland, OH) and thermally modified maize starch (DDWM, Cerestar, Vilvoorde, Belgium) with or without miconazole nitrate. In the case of tablets loaded with miconazole nitrate, the tablets consisted of 10.0 mg miconazole nitrate (Sigma Chemical Co., St. Louis, MO), 82.8 mg DDWM, 5.0 mg polyacrylic acid, 2.0 mg sodium benzoate (<90 μm, Flandria, Zwijnaarde, Belgium), and 0.2 mg silicium dioxide (<90 μm, Aerosil 200, Pharmachemic, Antwerpen, Belgium). For the tablets without miconazole nitrate, they consisted of 95.0 mg DDWM and 5.0 mg polyacrylic acid. The tablet formulations differed in the type of polyacrylic acid that was used: Carbopol 907 (MW 450,000), Carbopol 910 (MW 750,000), and Carbopol 934 (MW 3,000,000). The powders were blended for 10 min in a Turbula mixer (Type T2A, W. A. Bachofen, Basel, Switzerland). Tablets of 100 mg were directly compressed at a pressure of 150 MPa on an eccentric compression machine (Korsch, Type EKO, Frankfurt, Germany), equipped with 7-mm flat punches. The tablets were 2 mm thick. Content uniformity of the tablets revealed an average (n = 10) of 100.6 ± 3.7, 100.2 ± 5.2, and 99.7 ± 4.8 of the theoretical amount of miconazole nitrate for the formulations containing Carbopol 907, 910, and 934, respectively.

In Vitro–in Vivo Correlation

The detachment force and work of adhesion of the tablets loaded with miconazole were previously evaluated *in vitro* by Bouckaert and Remon (8). The apparatus used for the determination of the bioadhesive characteristics consisted of a tensile testing machine (Type L1000R, Lloyd Instruments, Segentworth, Fareham), equipped with a 20-N load cell. The measurements were performed in an isotonic buffered solution, pH 7.4 (2.38 g $Na_2HPO_4 \cdot 2H_2O$, 0.19 g KH_2PO_4, and 8.0 g NaCl made up to 1000 mL with demineralized water, all reagents p.a. grade).

In the first study a comparison of the formulations con-

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[4] A patent for the application of modified starch as a bioadhesive slow-release drug carrier (E.P. No. 9087005.2-) has been applied for.

0724-8741/93/0600-0853$07.00/0

taining 5% Carbopol 934 and 907 loaded with miconazole nitrate was performed in 10 healthy volunteers (7 male and 3 female, ages ranging from 20 to 41 years). In the second study, to compare the formulations containing 5% Carbopol 907 and 910 loaded with miconazole nitrate, eight healthy volunteers (six male and two female, ages ranging from 23 to 42 years) participated. Both studies had a randomized blind crossover design. Informed consent from the volunteers and the approval of the ethics committee of the Gent Medical School was obtained. Good Clinical Practice rules were applied. An interval of at least 4 days was respected between the different tablet administrations.

The volunteers were instructed to finish breakfast (consisting of white bread, butter and/or jam, coffee, tea or milk) no later than 8.00 AM. Thirty minutes later, the bioadhesive tablet was administered. A standard meal was given in the period of 305 until 335 min after administration of the tablet. The volunteers were allowed to take a nonstandard supper after sampling at 540 min. During the experiment the volunteers were allowed to drink water ad libitum from 60 min after administration of the tablet. No drinking was allowed 10 min before collection of the saliva samples. The volunteers were asked to record their remarks regarding their experience with the tablet. The tablet was placed on the attached gingiva in the region of the right upper canine and fixed for 1 min with a slight manual pressure on the lip. Next the tablet was moistened with the tongue to prevent sticking of the tablet to the lip. The adhesion time of the tablet was noted and was defined as the time after which the bioadhesive tablet was no longer visible under the lip upon control at 30-min interval.

Prior to the application of the formulation, a blank saliva sample was taken. Saliva samples were then taken 15, 30, 60, 120, 180, 240, 300, 420, 540, and 660 min after application. Two milliliters of saliva was collected over a 2-min period (1 min before and 1 min after the given time) in a borosilicate tube (16 × 125, Corning, New York). Care was taken that the tongue did not contact the tablet during the 10 min before sampling, in order to avoid abnormally high drug levels. The saliva samples were stored at −20°C pending analysis. The miconazole concentration in saliva was determined using a HPLC method as described by Bouckaert *et al.* (9).

In Vivo Evaluation of the Adhesive Behavior of Tablets Formulated Without Drug

The *in vitro* bioadhesive characteristics of the tablets consisting of a mixture of DDWM and 5% polyacrylic acid (PAA 907, 910, 934) were previously described (8). The adhesion time and the behavior of these tablets were now determined in a blind crossover study with seven healthy volunteers (six male and one female, ages ranging from 20 to 42 years). The volunteers received the tablet in numbered vials. They were instructed to test one tablet per day, with an interval of 2 days, and to insert the tablet in the morning at 8 AM after breakfast. The tablet was placed on the attached gingiva in the region of the right upper canine and fixed for 1 min with a slight manual pressure on the lip. Next the tablet was moistened with the tongue to prevent sticking of the tablet to the lip. The volunteers were asked to record the time of adhesion and the circumstances of the end point of adhesion (erosion or detachment of the tablet) by control at 30-min intervals.

Data Analysis

Results are given as mean ± SD. The *in vitro* results, taken from Bouckaert and Remon (8), were statistically evaluated using a one-way analysis of variance (10). The maximal salivary concentration (C_{max}) of miconazole and the time to reach the maximal salivary miconazole concentration (T_{max}) were determined from the concentration–time curves. The area under the curve (AUC) was calculated using the ABSPLOTS program (11). The adhesion time, AUC, C_{max}, and T_{max} values were statistically evaluated using nonparametric statistics (12).

RESULTS AND DISCUSSION

In Vitro–in Vivo Correlation

The results of the *in vitro* bioadhesion tests are shown in Table I. The formulation containing 5% Carbopol 907 had a detachment force that was not significantly different ($P > 0.05$) from the formulation containing Carbopol 934, while the work of adhesion was significantly higher ($P < 0.001$). The formulation containing Carbopol 907 had a similar work of adhesion, but a significantly ($P < 0.05$) lower detachment force in comparison with the formulation containing Carbopol 910. Therefore, two correlation studies were performed. In the first study, a comparison was made between the tablets, containing miconazole nitrate and formulated with Carbopol 907 and 934. Both bioadhesive tablet formulations were well accepted by the volunteers and no irritation was recorded. All tablets eroded completely; in two volunteers the tablet had disappeared after the standard lunch (one with Carbopol 907 and one with Carbopol 934), probably by swallowing. The mean adhesion time for the formulation with Carbopol 907 was 537 ± 133 min (range, 330–870 min) and was not significantly ($P > 0.05$; two-tailed Wilcoxon test) different from that for the formulation with Carbopol 934, with an adhesion time of 555 ± 182 min (range, 300–870 min) (Table IIa). The salivary miconazole levels obtained after application of both bioadhesive tablets are shown in Fig. 1. The individual release characteristics of the two tablets were not significantly different as judged by the AUC, C_{max}, and T_{max} parameters (Table IIa) ($P > 0.05$). These results indicated that although the work of adhesion for the formulation containing Carbopol 907 is twice the value obtained for the formulation containing Carbopol 934, no sig-

Table I. Detachment Force and Work of Adhesion for the Formulations Consisting of Drum-Dried Waxy Maize Starch and 5% of Three Types of Polyacrylic Acid with 10 mg Miconazole Nitrate (Results Taken from Bouckaert and Remon, 1992); Mean Values ± SD

	n	Detachment force (N)	Work of adhesion (mJ)
PAA 907	10	2.783 ± 0.337	1.011 ± 0.218
PAA 910	11	3.341 ± 0.569*	1.015 ± 0.218
PAA 934	10	2.518 ± 0.582	0.590 ± 0.210*

* Significant different versus PAA 907.

Table II. Mean ± SD Adhesion Time, AUC, C_{max}, and T_{max} Values for the Miconazole Containing Bioadhesive Tablet Formulated with 5% Carbopol 907 (PAA 907) and Carbopol 934 (PAA 934) in the First Correlation Study (n = 10; a) and with 5% Carbopol 907 (PAA 907) and Carbopol 910 (PAA 910) in the Second Correlation Study (n = 8; b)

	Adh. time (min)	AUC ($\mu g \cdot min \cdot mL^{-1}$)	C_{max} ($\mu g \cdot mL^{-1}$)	T_{max} (min)
		a		
PAA 907	537 ± 133	25.0 ± 4.5	109 ± 20	234 ± 99
PAA 934	555 ± 182	26.9 ± 9.9	114 ± 30	204 ± 58
		b		
PAA 907	603 ± 104	27.3 ± 7.5	115 ± 38	315 ± 131
PAA 910	600 ± 174	25.4 ± 7.6	109 ± 22	210 ± 55

nificant difference was observed in the biopharmaceutical parameters and the adhesion time *in vivo* between the two formulations.

For the second study on tablets containing miconazole nitrate, a comparison was made of the formulations containing Carbopol 907 and 910. The salivary miconazole levels are shown in Fig. 1 and the calculated parameters in Table IIb. Both bioadhesive tablets were well accepted by the volunteers and all tablets eroded completely in the buccal cavity, except in two volunteers (one volunteer with the formulation containing Carbopol 907 and one with the formulation containing Carbopol 910), where the tablet disappeared after the standard lunch. No tablet had to be removed due to irritation. The mean adhesion time for the tablet containing Carbopol 907 was 603 ± 104 min (range, 390–720 min) and was not significantly ($P > 0.05$; two-tailed Wilcoxon test) different from that for the formulation containing Carbopol 910, with a mean adhesion time of 600 ± 174 min (range, 300–870 min). All the parameters (Table IIb) indicated that although the detachment force *in vitro* was significantly ($P < 0.05$) different for Carbopol 907 compared to Carbopol 910, with a similar work of adhesion, no significant ($P > 0.05$; two-tailed Wilcoxon test) difference could be seen *in vivo* between both formulations.

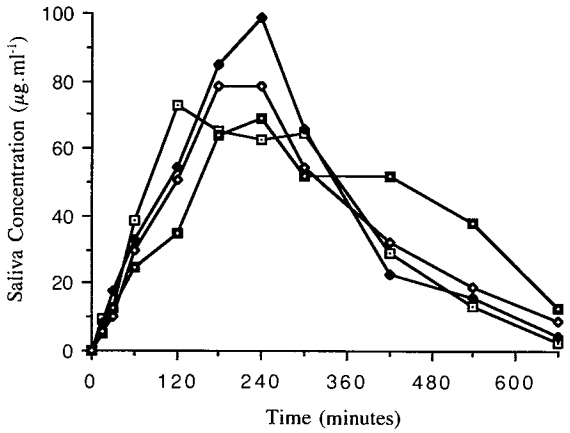

Fig. 1. Mean salivary miconazole concentrations after administration of the bioadhesive tablet formulated with Carbopol 907 (—▣—) and Carbopol 934 (—◆—) in the first correlation study (n = 10) and with Carbopol 907 (—▣—) and Carbopol 910 (—◆—) in the second correlation study (n = 8).

Both studies indicated that although a difference was observed *in vitro* between formulations, this difference was not reflected in adhesion time or in biopharmaceutical parameters calculated *in vivo*. It can be concluded that it is unclear which of both *in vitro* bioadhesive parameters is important for the *in vivo* performance of buccal bioadhesive tablets.

Seven volunteers participated in both correlation studies. We were thus able to evaluate the reproducibility of the adhesion time and the biopharmaceutical parameters of the tablet, based on a DDWM/polyacrylic acid 907 mixture containing miconazole nitrate. The adhesion time in these seven volunteers was 566 ± 136 and 600 ± 112 min in the first and second study, respectively. Values were 25.2 ± 5.2 and 28.6 ± 7.1 mg · min · mL^{-1} for AUC, 109.9 ± 23.9 and 121.9 ± 37.4 $\mu g \cdot mL^{-1}$ for C_{max}, and 257 ± 96 and 334 ± 128 min for T_{max}. These values were not significantly different ($P > 0.05$; two-tailed Wilcoxon test), illustrating the acceptable reproducibility of the biopharmaceutical behavior of the tablet.

In Vivo Evaluation of the Adhesive Behavior of Tablets Formulated Without Drug

In order to confirm our previous findings and to elucidate the meaning of the *in vitro* bioadhesive characteristics, we performed an *in vivo* study with tablets consisting of 5% polyacrylic acid (PAA 907, 910, 934) and DDWM, but without miconazole nitrate. The formulation also does not contain 2% sodium benzoate and 0.2% silicium dioxide. The results of the *in vitro* bioadhesive determinations are shown in Table III. Considering these *in vitro* results we can conclude that the same trends are seen as for the formulations containing miconazole nitrate and that no significant influence was noticed when the drug was added to the polymers. *In vivo,* all tablets were well accepted by the volunteers and no tablet had to be removed due to irritation. All tablets eroded completely. The mean adhesion times were (n = 7) 814 ± 107 (range, 630–960), 791 ± 144 (range, 630–1020), and 782 ± 191 (range, 570–1020) min for the formulations containing Carbopol 907, 910, and 934, respectively (Table IV). No significant ($P > 0.05$; Friedman two-way analysis of variance) difference could be observed between the tablet formulations, confirming our previous findings with the drug-loaded polymers. Surprisingly, the adhesion times were much higher than those obtained for the formulations loaded

Table III. Detachment Force and Work of Adhesion for the Formulations Consisting of Drum-Dried Waxy Maize Starch and 5% of Three Types of Polyacrylic Acid Without 10 mg Miconazole Nitrate (Results Taken from Bouckaert and Remon, 1992); Mean Values ± SD

	n	Detachment force (N)	Work of adhesion (mJ)
PAA 907	10	2.708 ± 0.573	1.154 ± 0.212
PAA 910	11	3.286 ± 0.702*	1.185 ± 0.296
PAA 934	10	2.621 ± 0.562	0.549 ± 0.132*

* Significant different versus PAA 907.

Table IV. Mean ± SD Adhesion Time for the Bioadhesive Tablet Consisting of 95% DDWM and 5% of Different Types of Polyacrylic Acid—Carbopol 907, 910, and 934—in Seven Volunteers

	Adhesion time (min)
PAA 907	814 ± 107
PAA 910	791 ± 144
PAA 934	782 ± 191

with miconazole nitrate. Although the *in vitro* method pointed out that the incorporation of 10% miconazole in the tablet did not induce a significantly lower detachment force and work of adhesion (8), a significantly ($P < 0.05$; Mann–Whitney U test) longer adhesion time was observed for the formulation without drug. The addition of 10% miconazole to the tablet formulation must thus have induced a decreased resistance to erosion in comparison to the tablet made of pure polymers. Therefore an *in vitro* method for the assessment of erosion may be necessary for optimization of a buccal bioadhesive dosage form.

The *in vitro* bioadhesion test seemed to provide information only on the initial adhesion, and not on the residence time of the tablet in the oral cavity. It is unclear which parameter, detachment force or work of adhesion, has to be considered when evaluating buccal bioadhesion. Care has to be taken when classifying polymers according to their *in vitro* bioadhesion properties, because differences seen *in vitro* do not necessarily induce significant differences *in vivo*. Further studies are needed to determine minimum values of detachment force and work of adhesion to achieve good bioadhesion.

ACKNOWLEDGMENTS

This study was financially supported by a grant from the Investigation Fund of the University of Gent (RUG 01133290).

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