Buccal Absorption of Testosterone and Its Esters Using a Bioadhesive Tablet in Dogs

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Purpose. As the oral bioavailability of testosterone is very low because of its high first pass effect, buccal administration might present a viable alternative. In this study a buccal bioadhesive tablet was used in order to sustain the delivery and bypass the liver.

Methods. Testosterone and testosterone acetate, propionate, enanthate and decanoate were investigated. The influence of the concentration of testosterone (10–50%) and testosterone esters (30%) on in vitro bioadhesion was investigated. The absolute (IV) and relative (oral) bioavailability of 60 mg testosterone or an equivalent amount of testosterone ester was determined in castrated male dogs.

Results. Both the in vitro detachment force and the work of adhesion decreased gradually with an increasing amount of testosterone and for an increasing chain length of the esters, except in the case of testosterone enanthate. The in vivo results revealed that the bioavailability of testosterone was significantly higher ($p < 0.05$) than that of the esters, which is probably due to the lower solubility of the esters. The mean absolute bioavailability of testosterone from the bioadhesive tablet was 14.1%, while the mean relative bioavailability was 1370%. The buccal administration of testosterone via the bioadhesive tablet allowed the maintenance of the plasma level at above 3 ng/ml for 15 to 24 h.

Conclusions. Buccal absorption of testosterone was significantly higher than that of its esters.

KEY WORDS: bioadhesion; buccal absorption; testosterone; dog model; bioavailability; in vitro bioadhesion.

INTRODUCTION

Testosterone, when administered orally or parenterally, is rapidly absorbed and metabolized by the liver, resulting in a very short circulating half life. Nowadays, testosterone esters are injected intramuscularly (e.g., testosterone propionate, enanthate and decanoate). They are more lipophilic than testosterone and are absorbed slowly when injected as an oil solution and suspension. Testosterone undecanoate is an orally active ester, whose lymphatic absorption is favoured by its formulation in oleic acid. Other routes of administration have been investigated, e.g., transdermal, nasal, sublingual, buccal and intramuscular administration and subcutaneous implantation (1–4). Recently the need for a sustained testosterone plasma level with a concentration which is above 3 ng/ml for men has been defined, requiring continuous testosterone input (2). Current therapies are unable to simulate the circadian rhythm of testosterone plasma levels in healthy men (1). Montanini et al. (5) showed that there was a loss of rhythmicity of testosterone levels in elderly men and that this could play an important role in developing functional and/or organic disturbances. Mazer and co-workers (2) were able to mimic the normal testosterone profile in hypogonadal men with the use of a transdermal patch. An alternative administration method is the buccal absorption of testosterone and its esters, as already described by Beckett and Pickup (4). These investigators found that testosterone acetate was absorbed faster than testosterone. This paper describes the buccal absorption of testosterone acetate, propionate, enanthate and decanoate from an erodible bioadhesive tablet.

MATERIALS AND METHODS

Materials

Testosterone and its propionate were purchased from Diosynth (Oss, The Netherlands). Testosterone acetate was obtained from Sigma Chemical Co. (St. Louis, USA). Testosterone enanthate was kindly supplied by Schering AG (Berlin, Germany) and testosterone decanoate was a gift from Organon International bv (Oss, The Netherlands). Drum Dried Waxy Maize (DDWM) was supplied by Eridiana Béghin Say Cerestar (Vilvoorde, Belgium). Carbopol 974P was supplied by BF Goodrich (Brussels, Belgium). Sodium stearyl fumarate (NaSF) was given by Edward Mendell Co. Inc. (New York, USA). All other chemicals used to prepare the buffered saline, were of analytical reagent grade.

Production of the Tablets

The powders were mixed in a Turbula mixer (Type T2A, W.A. Bachofen, Basel, Switzerland) for 10 minutes. Next the sodium stearyl fumarate was added as a lubricant and mixed in for an additional 5 minutes. The tablets (200 mg) used for the in vivo study were compressed on a Korsch compression machine (Type EKO, Berlin, Germany) equipped with 9 mm flat punches, with a pressure of 14.7 kN. The testosterone ester tablets were prepared in a similar way so that an equivalent amount of 60 mg testosterone was formulated. The composition of the formulations is shown in Table I.

The weight and the diameter of the tablets used for the bioadhesion measurements was 100 mg and 7 mm, respectively.

Table I. Testosterone and Testosterone Esters with Their Log P Values (Octanol/Water) and Solubility in Water

Drug	Log P[a]	Solubility at 37°C in water ($\mu g.mL^{-1}$)
Testosterone	3.219	46.3[b]
Testosterone acetate	4.165	8.5[b]
Testosterone propionate	4.694	3.9[b]
Testosterone enanthate	6.810	not soluble
Testosterone decanoate	8.397	not soluble

[a] Calculated (Ref 16).
[b] Ref 17.

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In Vitro Determination of Bioadhesion

The bioadhesion of the tablets was evaluated according to a previously described method (6). The detachment force and the work of adhesion were determined as the height and the area under the curve of the force vs extension diagram. The apparatus consisted of a tensile testing machine (type L1000R, Lloyd Instruments, Segenworth, Fareham, UK), equipped with a 20 N load cell. Porcine gingiva were obtained at the slaughter house where they were excised directly after slaughtering. The mucosa were stored at −20°C in isotonic buffered saline pH 7.4 (2.38 g $Na_2HPO_4H_2O$, 0.19 g KH_2PO_4, 8.0 g NaCl made up to 1000 ml with demineralized water).

The porcine gingival tissue was attached with cyanoacrylate glue (Loctite, Belgium) to a lower Teflon support, while the tablet was attached to an upper aluminium punch. After hydrating the mucosa with 15 µl of the isotonic phosphate buffered saline, the tablet was fixed on the mucosa applying a force of 0.5 N for 5 min. After the initial contact, the beaker was filled with 125 ml isotonic buffered saline pH 7.4. Next, the tablet and mucosa were pulled apart at a speed of 5 $mm.min^{-1}$ until a complete rupture of the tablet-mucosa bond was obtained. The influence of the testosterone concentration (10–50%, w/w) on the bioadhesion was investigated compared with a tablet containing only the excipients. The testosterone esters were used in a concentration of 30% (w/w). Statistical analysis was performed using a one-way analysis of variance at a significancy level of $p < 0.05$ ($n = 6$) (7).

In Vivo Studies

The bioavailability of all formulations listed in Table II was determined in 6 castrated male dogs (weight 32.8 ± 6.4 kg). The dogs were conscious and fasted from 12h before until the end of the experiment. Drinking water was available at libitum. One tablet was placed on the gingiva above the right upper canine and blood samples were collected before the administration and 0.5, 1, 2, 4, 8, 12, 16, 24h after the administration in heparinized tubes. The same sampling times were respected after the oral administration of 60 mg testosterone, administered in a hard gelatin capsule. The blood samples were centrifuged at 2000 g and the plasma was kept at −20°C until analysis. A time interval of at least 1 week was respected between each administration. In order to investigate the absolute bioavailability, 60 mg testosterone was administered intravenously to each dog. Blood samples were taken at 0, 2, 5, 10, 20, 30 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24h after administration. Testosterone was isolated from plasma by ether extraction (20 ml ether / 2 ml plasma). The ether extract was evaporated until dryness under a stream of nitrogen and the residue dissolved in 0.5 ml phosphate buffer (0.01 M, pH 7.4, containing 0.125 M sodium-azide and 1 g/L BSA). Testosterone concentrations were determined by radio-immunoassay using a testosterone-antiserum raised in the goat by treatment with testosterone-3-O-carboxy-methyl-oxime:BSA (testosterone-CMO-BSA) (^{3}H-Testosterone RIA-kit, BioMérieux, Marcy L'Etoile, France) (8). The antiserum was highly specific for testosterone, the interference from other steroids (eg. androstene-dione) being less than 1%, while the inter- assay variation coefficient of the estimation method was 6.5%.

The absolute (IV) and relative (oral administration) bioavailability was calculated using the Kinbes® software (9). The AUC was calculated with a mixed integration algorithm. The $T_{>3ng/ml}$ value (time during which the concentration was above 3 ng/ml) was calculated from the individual graphs. Statistical analysis of the results was performed using the Wilcoxon singed rank test ($p < 0.05$, $n = 6$) (10).

The adhesion time of the bioadhesive tablet was determined visually. Adhesion was considered to be present until the complete erosion of the tablet.

Table II. Composition of the Buccal Adhesive Tablets. The Amounts Are Given in w/w (%)

	Drug	Carbopol	NaSF[a]	DDWM[b]
Testosterone	30.0	5	1	64
T acetate	34.4	5	1	59.6
T propionate	35.8	5	1	58.2
T enanthate	41.7	5	1	52.3
T decanoate	46.2	5	1	47.8

[a] Sodium stearyl fumarate.
[b] Drum Dried Waxy Maize.

RESULTS

The results of the in vitro bioadhesion determinations are shown in Fig. 1. The results of the bioadhesion measurements of the control tablet are in good agreement with the data published by Bouckaert et al. (6). The control formulation showed the highest mean detachment force and work of adhesion, although there was no significant difference with the tablet containing 10% testosterone. Both the work of adhesion and the detachment force decreased gradually with an increasing amount of drug. The tablets containing a testosterone concentration above 10% showed a significantly lower bioadhesion

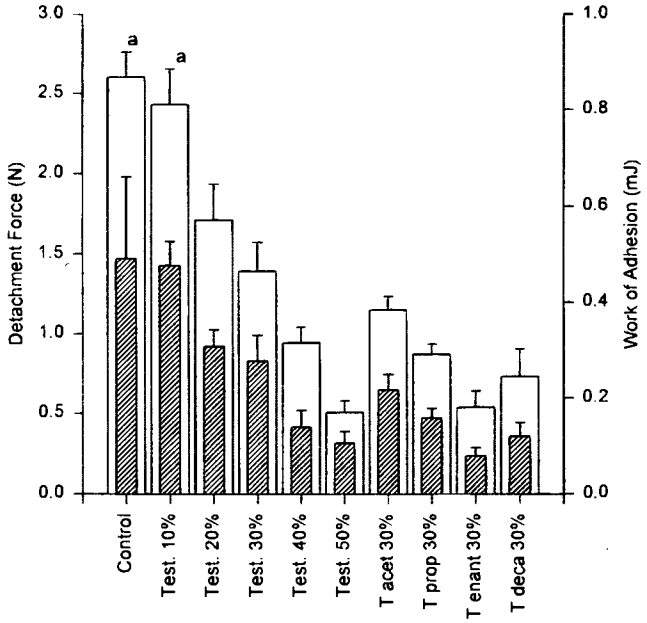

Fig. 1. Results of the bioadhesion measurements showing the two parameters detachment force (N) □ and work of adhesion (mJ) ▨, for each formulation. [a] significantly higher than all other formulations.

($p < 0.05$) in comparison with the control formulation. The tablets containing 30% testosterone esters showed a significantly lower value for both bioadhesive parameters in comparison with the tablet formulated with 30% testosterone. Although the same concentration was used for all the esters, the bioadhesion decreased gradually with an increasing chain length of the esters, except for testosterone enanthate. During the adhesion tests the tablets containing the enanthate ester split when the tablet was pulled from the mucosa, while the lower part of the tablet remained on the mucosal surface. A plausible explanation for this phenomenon was the melting temperature of testosterone enanthate (37°), being equal to the temperature of the buffer solution used in the adhesion test.

Fig. 2 and 3a-d show the plasma concentration time profiles of the different formulations. The bioavailability after oral administration of 60 mg testosterone in comparison with buccal administration was very low (Fig. 2, Table III). The absolute bioavailability of orally administered testosterone was only 1.03% (±0.75), whereas the absolute bioavailability of testosterone after buccal administration of the bioadhesive tablet was 14.14% (±7.21) (Table III). The AUC value after the buccal administration of testosterone (220.4 ± 134.8 h.ng.mL^{-1}) was significantly ($p < 0.05$) higher than the AUC values after the administration of all formulations containing the testosterone esters and of orally administered testosterone. All the formulations containing testosterone esters showed a very low absolute bioavailability (below 3%) (Table III). These findings were also reflected in the time during which the plasma concentration was above 3 ng/ml ($T_{>3ng/ml}$). Only the bioadhesive tablet containing 60 mg testosterone was able to sustain the target concentration for 20 h (15–24h). The $T_{>3ng/ml}$ values for the testosterone ester formulations were all lower than 10 h. Some individual plasma profiles after the administration of the testosterone esters did not reach the target concentration of 3 ng/ml, whereas the minimum $T_{>3ng/ml}$ value for the formulation containing testosterone was 15 h.

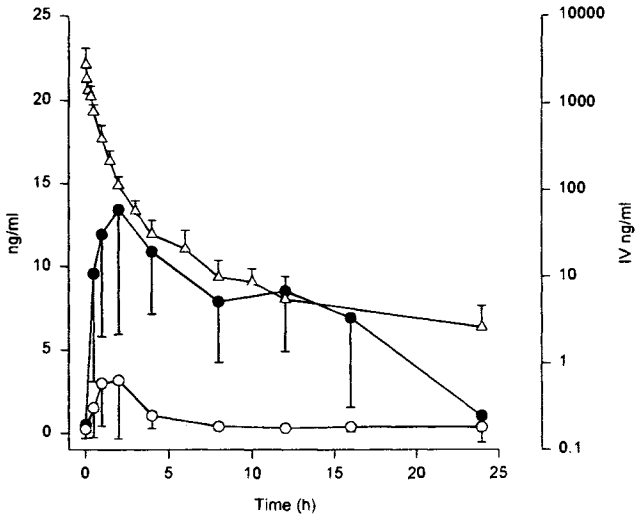

Fig. 2. Plasma concentration time profiles (mean values ± s.d.; n = 6) after buccal (–●–) and oral administration (–○–) of 60 mg testosterone and after intravenous administration (–△–) of 60 mg testosteron.

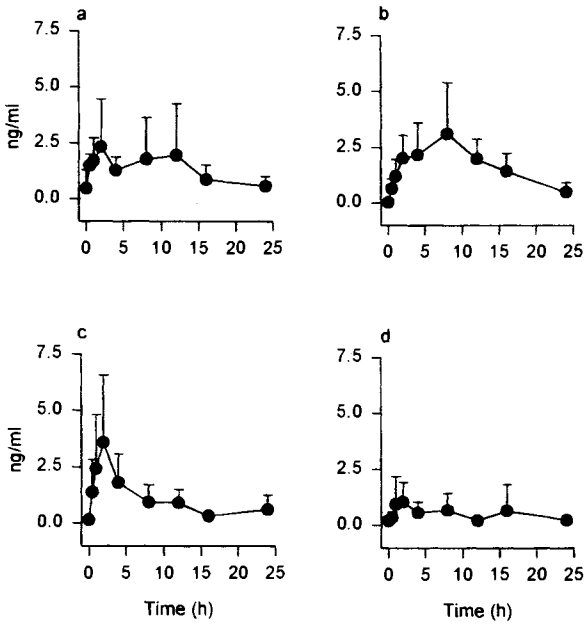

Fig. 3. Plasma concentration time profiles (mean values ± s.d.; n = 6) after buccal administration of the different testosterone esters; a: Testosterone acetate; b: Testosterone propionate; c: Testosterone enanthate; d: Testosterone decanoate.

DISCUSSION

The use of bioadhesive devices for local and systemic drug delivery has been extensively investigated. The oral mucosa is easily accessible for the delivery of drugs and, with the use of a slow release bioadhesive tablet, sustained plasma levels can be achieved. The tablet used in this study has already shown to be effective in the buccal delivery of locally active drugs such as miconazole nitrate (11). In humans it was reported that the gingiva was the best application site of the tablet for local therapy, compared to the palate and the cheek (12). The tablet applied to the gingiva showed the longest adhesion time. In this study, where dogs were used as a model, the tablet was also applied to the gingiva. The canine tissue appears to compare very well with human buccal tissue, especially with respect to the structure and blood flow (13). For diclofenac a correlation has been reported between human and canine buccal drug absorption (13,14). In this study none of the tablets detached from the gingiva before complete erosion, even when they were formulated with a high dose of testosterone ester. The tablet containing testosterone enanthate eroded faster in comparison with the other formulations. The tablet spread on the mucosa within 15 min after application and eroded progressively. This tablet also showed the lowest mean adhesion time in vivo (Table III) and performed poorly during in vitro bioadhesion experiments. No correlation was found between the in vitro bioadhesion data and the mean in vivo adhesion time for all other formulations investigated. The absolute bioavailability of orally administered testosterone was very low (1.03 ± 0.75%). This is in accordance with the findings of Johnsen et al. (15), who studied the therapeutic effectiveness of orally administered testosterone in men and reported that 100 mg testosterone per day was ineffective and that 400 mg was needed for a therapeutic effect. In our study

Table III. Pharmacokinetic Data. All Values Are Expressed as a Mean ± s.d. (n = 6). The $T_{<3ng/ml}$ Is Given as a Min-Max Range Value

	AUC (h.ng.mL^{-1})	$T_{>3ng/ml}$ (h)	F_{ABS} (%)	F_{REL} (%)	Mean adhesion time (h)
Oral	16.1 ± 10	0 – 3h	1.03 ± 0.75	1	—
Testosterone	220.4 ± 134.8[a]	15 – 24h	14.14 ± 7.21	1370 ± 1208	11.33 ± 2.16
Testosterone acetate	29.2 ± 18.9	0 – 9h	1.87 ± 1.27	181.3 ± 128.3	14.75 ± 8.29
Testosterone propionate	42.6 ± 12.4	0 – 8h30	2.73 ± 0.82	264.8 ± 212.2	12.66 ± 2.25
Testosterone enanthate	24.9 ± 13.6	0 – 4h	1.60 ± 0.92	154.8 ± 116.9	9.00 ± 5.36
Testosterone decanoate	12.8 ± 10.7	0h	0.82 ± 0.61	79.7 ± 86.6	15.66 ± 2.27

[a] Significantly higher ($p < 0.05$) than all other AUC's.

the bioavailability of the buccally administered testosterone in dogs was higher (14.14 ± 7.21%). This observation proved that the plasma concentration profile after the buccal administration of testosterone was almost completely due to buccal absorption. A plausible explanation for the still low bioavailability after buccal administration is the fact that an erodible tablet without any backing layer was used and that the loss of drug due to swallowing could not be prevented. The fraction of testosterone that was swallowed was metabolised as a result of the first pass effect. A major advantage of the bioadhesive erodible tablet is the availability of a larger buccal mucosal surface for the absorption of the drug, especially when higher amounts of the drug need to be absorbed.

Although the esters have a higher lipophilicity compared to testosterone, they did not show a higher bioavailability. These findings are not in agreement with the data of Beckett and Pickup (4), who calculated a higher absorption rate constant and a higher percentage buccally absorbed testosterone acetate in comparison with testosterone. They reported a high correlation between absorption and lipophilicity, but used a drug solution in their experiment which was circulated in the mouth for a given time. In this study the steroids were formulated as a bioadhesive tablet. The lower solubility of the testosterone esters in comparison to testosterone seemed to decrease the bioavailability dramatically. The low solubility of the esters could explain that an important fraction of the drug was swallowed before being available for absorption. The high lipophilicity of the testosterone esters could also have a negative effect on the absorption, regardless of their low solubility. Wils et al. (18) studied the transport across intestinal cells (HT 29-18-C_1 and Caco-2) of a series of drugs with log $D_{o/b}$ values (octanol/buffer distribution coefficient) ranging from −3 to more than 5. For molecules with a log $D_{o/b}$ value above 3.5 they reported a decrease in the in vitro permeability coefficient when lipophilicity increased. The "cut-off" of the $D_{o/b}$ value appeared to be around 3000. As the log P value of testosterone is 3.219, it seems clear that testosterone has an optimal lipophilicity for intestinal absorption. According to their theory the testosterone esters have a log P value which is too high to yield a high bioavailability. Although the study of Wils et al. (18) was performed with intestinal cells, both the intestinal and the buccal cells are epithelial tissues with a mucus layer (only the HT29-18-C_1). Beckett and Pickup (4) suggested that the absorption of steroids from the buccal cavity took place by partitioning into the surface epithelial cells followed by a diffusion across the membrane into the blood circulation. In addition to the dramatically higher surface of intestinal tissue due to the presence of microvilli, the mucosa in the buccal cavity are partially keratinized (gingiva and hard palate) which might affect drug absorption. Garren and Repta (19) found a linear relationship between the log epithelial permeability and the log of the octanol-buffer partition coefficient using the keratinized hamster cheek pouch model. They used substituted acetanilides with log P values ranging from 0.91 to 2.73 and stayed below the "cut off" point of 3000 mentioned by Wils et al. (18). But unless a proper correlation with non-keratinized tissue has been found, data from rodent buccal tissue should be interpreted with caution.

This study shows that the application of a buccal bioadhesive tablet with 60 mg testosterone in dogs sustained plasma levels which were significantly higher than those obtained after the oral administration of the same dose of testosterone. The buccal absorption of testosterone esters was significantly lower than the buccal absorption of testosterone. The correlation between the data obtained in dogs and human buccal absorption of testosterone still has to be proved.

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