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Biokinetics of buccal spray insulin in patients with type 1 diabetes

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Abstract

Objective: To evaluate the metabolic effect of buccal spray insulin compared with subcutaneous regular insulin in patients with type 1 diabetes.
Research Design and Methods: This study compared plasma glucose, insulin, and C-peptide levels in 18 patients with type 1 diabetes treated with subcutaneous regular or buccal spray insulin on 2 consecutive mornings. On day 1, patients were treated with their usual subcutaneous regular insulin regimens. On day 2, patients received buccal spray insulin. In the morning of both days 1 and 2, patients received a standard meal of 630 kJ. No intermediate or long-acting insulin was administered to patients on the morning of the test. Blood samples were collected for up to 4 hours for biokinetic analysis. In a subset of 3 patients, premeal buccal spray insulin was administered for 2 entire consecutive days. In these patients, glucose levels were monitored using the glucose sensor monitoring system.
Results: Overall, there were no statistically significant differences in glucose, insulin, or C-peptide levels measured after administration of subcutaneous vs buccal spray insulin. However, at 90 and 120 minutes after subcutaneous regular insulin administration, significantly higher insulin levels *and more prolonged hypoglycemic effect* were detected compared with buccal spray insulin administration. In the 3 patients who received 1 day of regular and 2 entire days of buccal spray insulin, no significant differences were observed in glucose levels during the 3 days of glucose sensor monitoring.
Conclusions: Insulin administered via the buccal spray formulation is as effective as the subcutaneous route in lowering blood glucose levels.

1. Introduction

Since the time of its discovery, insulin has been administered to diabetic patients via injection. To achieve therapeutic targets, which are known to reduce the risk of long-term diabetic complications [1], a tight control of blood glucose levels is required that often necessitates multiple daily insulin injections [2]. This therapeutic protocol is recognized to be inconvenient especially in young patients, and therefore, many efforts have been made to identify new ways of delivering insulin.

Native insulin is a protein hormone with a molecular weight of 5900 and, as such, is not absorbed by the gastrointestinal mucosa because it gets degraded by proteolytic enzymes present throughout the gastrointestinal tract. Several attempts to overcome barriers to insulin *absorption in the gastrointestinal mucosa* have been made in the past with very little success. With the application of new technologies, alternative means of insulin delivery have emerged to avoid or at least reduce the need for patients to be treated with multiple daily injections. One of the possible alternative routes of insulin administration is through the buccal mucosa because this site is highly vascularized and acceptable for drug administration.

Recently, a new delivery system has been developed based on a formulation technology that allows liquid insulin to be delivered accurately into the mouth of patients [3]. This technology uses the formation of microfine, thin membrane, mixed micelles made from the combination of insulin and specific absorption enhancers that encapsulate and protect the insulin molecules. Because the system introduces a fine particle aerosol at high velocity into the patient's mouth, deposition and absorption of the pharmaceutical preparation are increased compared with conventional technology. The fast moving fine particle aerosol can quickly traverse the superficial layers of the buccal mucosa. Once through, insulin gets rapidly absorbed into the

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bloodstream with the aid of absorption enhancers and appears in the circulation within 10 minutes of application [3]. The buccal spray insulin formulation is packed into a small portable container that is attached to a canister. For insulin delivery, the canister is placed into the mouth and puffs are released by pressing on the device.

Buccal spray insulin (Oralin) was previously tested in healthy volunteers and was found to be absorbed by the buccal mucosa and to lower blood glucose levels [4,5]. Buccal spray insulin was also evaluated in patients with type 2 insulin-treated diabetes as meal time insulin therapy. It was found that buccal spray insulin was absorbed slightly faster than subcutaneous regular insulin injection [6].

In the present proof of concept trial, buccal spray insulin was administered to patients with type 1 diabetes to compare its efficacy in lowering blood glucose with that of subcutaneous regular insulin in response to a standard meal.

2. Patients and methods

Eighteen patients with type 1 diabetes (according to World Health Organization classification), 12 men and 6 women, with a mean disease duration of 6 years, glutamic acid decarboxylase antibody positive at diagnosis, volunteered for this study. Inclusion criteria stipulated patients to be between 18 and 50 years of age, show evidence of good metabolic control as assessed by hemoglobin A_{1c} values of less than 8%, and have an absence of diabetic complications or other cardiovascular, renal, or liver diseases. The clinical characteristics of these patients are shown in Table 1. These patients are typical, representing those with type 1 diabetes diagnosed above 18 years of age [7]. All patients were on intensive insulin therapy at the time of entry into the trial, with 3 injections of regular insulin at meal times and 1 NPH insulin injection at bedtime.

The protocol was approved by the Ethical Committee at University Campus Bio-Medico and all patients gave their written informed consent.

The study protocol consisted of tests run on 2 consecutive mornings. Briefly, on day 1, patients were treated with their standard dose of subcutaneous regular insulin followed by a meal to assess the amount of insulin required to control postprandial hyperglycemia. On day 2, patients received buccal spray insulin by means of puffs, producing an insulin availability equivalent to that of the subcutaneous insulin received the day before, followed by the same meal. To determine the correct insulin dose to be administered in buccal spray form, treatment randomization was not applied. Thus, in this proof of concept study, we ensured that the dose of buccal spray insulin to administer was as much as possible comparable with the one the patient needed to control blood glucose, and this is why we opted for this protocol and not for a randomized trial. Based on preliminary testing carried out in our center, the dose of buccal spray insulin required to reach normal blood glucose levels was approximately 5 to 7 times higher than insulin given by the subcutaneous route without any additional intermediate (NPH) insulin.

Table 1
Clinical and laboratory parameters of patients with type 1 diabetes entering the study

Age (y)	Disease duration (y)	BMI	Fasting C peptide (nmol/L)	Hemoglobin A_{1c} (%)	Daily insulin (IU/kg)
33.6 ± 10.5 (21-52)	6 ± 5.6 (1-12)	23 ± 2.0 (19-27)	0.2 ± 0.2 (0.02-0.33)	6.7 ± 1.3 (5.3-7.7)	0.41 ± 0.2 (0.1-1.3)

Values are expressed as mean ± SD (range). BMI indicates body mass index.

Specifically, on the morning of day 1 after an overnight fasting, patients received their dose of subcutaneous regular insulin 20 minutes before a standard meal of 630 kJ containing 55% carbohydrates, 15% protein, and 30% lipids (Ensure plus, Nutricia-Milan, Italy). The dose of subcutaneous regular insulin varied among patients from 5 to 20 IU. The procedure was the same on the morning of day 2 except that buccal spray insulin was administered. In terms of energy, this meal corresponds to the standard type of breakfast patients consume in Italy; therefore, standard living conditions on the occasion of the test were recreated.

Blood samples were collected every 30 minutes up to 2 hours and then every 60 minutes for another 2 hours to measure blood glucose, plasma insulin, and C-peptide levels. No basal insulin was administered to patients on the morning of the test; the last insulin injection was NPH insulin taken the night before. Therefore, the biokinetic and hypoglycemic action of the buccal spray insulin were evaluated without the effect of other insulin types.

In 3 male patients with type 1 diabetes (ages 28, 34, and 35 years), the pre-meal buccal spray insulin treatment was prolonged for 2 consecutive days. In these patients, glucose levels were monitored throughout the day for 3 consecutive days by the recently developed glucose sensor monitoring system [8]. On the first day, patients were treated according to their usual regimen of 3 preprandial regular insulin doses followed by bedtime NPH insulin. On the second day, the same patients switched to buccal spray insulin at meals with the addition of NPH insulin at bedtime. On the third day, the dose of buccal spray insulin was adjusted on the basis of glucose values reported the day before. In this respect, patients were taught how to adjust the dose of buccal spray insulin according to the postprandial glucose levels measured at home. If 2-hour past meal values were above 140 mg/dL, the number of puffs would have been increased by 20% in the third day at the corresponding meal.

In the blood samples collected during the meal test, plasma glucose was measured by standard laboratory method, whereas plasma insulin and C peptide were evaluated by radioimmunoassay.

2.1. Sample size

The number of patients included in the study was calculated considering a difference less than 15% in blood glucose at 1 and 2 hours between the 2 insulin treatments (subcutaneous vs oral) as “no difference.” Setting $\alpha = .05$

and β = 90%, the required sample size was 13 patients. We included 5 more patients in case of dropouts.

2.2. Statistical analysis

Data are expressed as mean ± SD and analyzed comparing results of the variables using the 2-tailed Student *t* test for paired data. In 5 randomly selected patients who received buccal spray insulin, we also calculated the intra-individual and interindividual variability of insulin absorption and plasma glucose values. The variation coefficient for each variable was calculated as the SD divided by the mean.

3. Results

Insulin administered via the buccal route was well tolerated by patients who completed the study. The biokinetic profile of subcutaneous regular insulin and buccal spray insulin is shown in Fig. 1A and B.

Blood glucose–lowering profiles during the meal test were not significantly different after administration of either subcutaneous regular or buccal spray insulin. Mean glucose values under the curve were slightly higher with buccal spray insulin compared with subcutaneous regular insulin at most points although no significant differences were observed even adjusting for multiple comparisons (Fig. 1A). These data probably reflect an under dose of insulin when buccal spray insulin was administered.

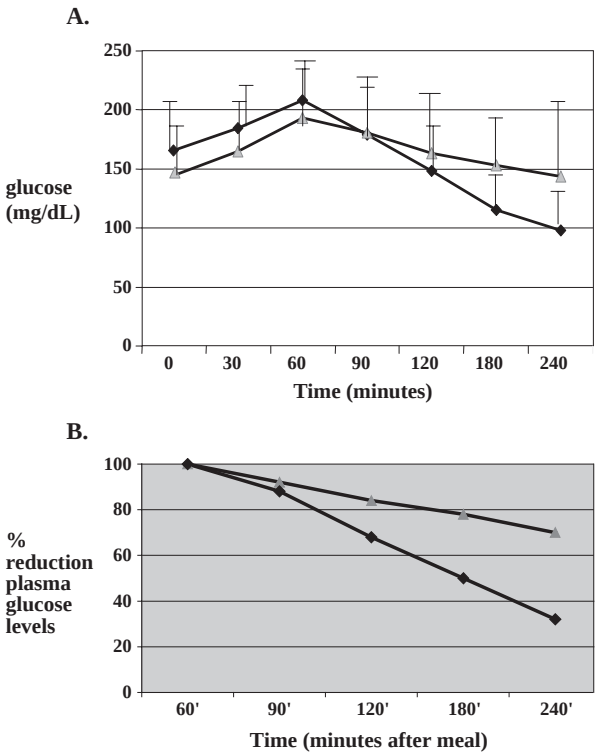

Fig. 1. A, Plasma glucose levels (mean ± SD) of patients receiving buccal spray insulin (—△—) or subcutaneous regular insulin (—◆—) after a standard meal. *P* = NS at each time point. B, Percentage of reduction in plasma glucose levels after buccal spray insulin (—△—) or regular subcutaneous insulin (—◆—) from time of 60 minutes after meal (180 minutes $P < .01$; 240 minutes $P < .001$).

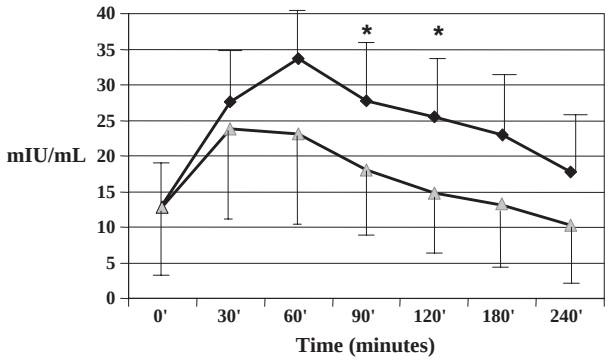

Fig. 2. Plasma insulin levels (mean ± SD) in patients receiving buccal spray insulin (—△—) or subcutaneous regular insulin (—◆—) after a standard meal and monitored with the glucosensor. $P < .008$ regular vs buccal spray insulin.

The duration of the hypoglycemic effect was more prolonged with regular insulin than buccal spay insulin, and at 180 and 240 minutes, the difference between the 2 insulin regimens was significant ($P < .01$ and $P < .001$, respectively). No episodes of severe hypoglycemia were detected with either insulin formulation.

Regarding plasma insulin levels, no significant differences were detected between the 2 insulin preparations (Fig. 2) except at 90 and 120 minutes where significantly higher plasma insulin levels were observed with subcutaneous regular insulin. T_{max} was reached at 30 minutes for buccal spray insulin and 60 minutes with subcutaneous regular insulin. However, it may still be possible that our proof of concept study was not powered enough to recognize a difference between a shorter action of buccal spray insulin vs regular insulin.

Regarding C-peptide levels, there was no stimulation of residual C-peptide secretion in individual patients or in the whole study group (Fig. 3), confirming that the hypoglycemic effect of buccal spray insulin was not due to stimulation of residual beta cell function.

In the 3 patients who received buccal spray insulin for 2 consecutive days and were monitored with the glucose

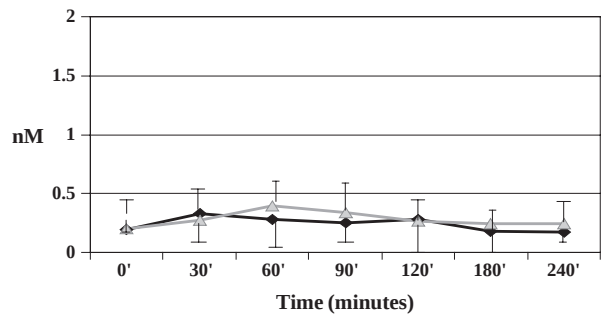

Fig. 3. Plasma C-peptide levels in patients receiving buccal spray insulin (—△—) or subcutaneous regular insulin (—◆—) after a standard meal. *P* = NS at each time point.

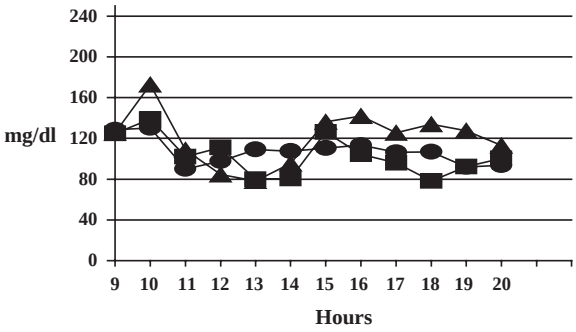

Fig. 4. Mean values of interstitial glucose in 3 patients with type 1 diabetes treated on separate days with buccal spray insulin or subcutaneous insulin at meal times + NPH at bed time. Subcutaneous regular insulin (–■–), buccal spray insulin day 1 (–▲–), and buccal spray insulin day 2 (–●–).

sensor, no significant differences were observed in plasma glucose levels between buccal spray or subcutaneous regular insulin treatments (Fig. 4).

Intraindividual variability of the insulin peak in 5 patients was 22% ± 20.2%, whereas intraindividual variability of the plasma glucose peak was 34.8% ± 28.6%. Interindividual variability of the insulin peak calculated in 4 patients was 21%. These values are similar to what was reported for regular insulin when administered by the subcutaneous route [9-11].

4. Discussion

This is the first study demonstrating that buccal spray insulin is effective in controlling postprandial glucose levels in patients with type 1 diabetes. Insulin absorption via the transmucosal route was shown to be reproducible with a mean intraindividual variability of 22% and with a peak of plasma insulin between 30 and 60 minutes after buccal administration. Regarding the intraindividual variability of buccal spray insulin, this was found to be similar to that observed with subcutaneous regular insulin. Thus, although we did not measure the intraindividual coefficient of variation of regular insulin in our trial, we can refer to other studies that reported an intraindividual coefficient of variation of 15% to 25% with regular insulin and even greater with long-acting insulin preparations (>50%) [10,11]. Insulin biokinetics was similar to the physiological postprandial peak of preformed insulin in healthy volunteers. Thus, in our study, this effect was clearly due to the buccal spray insulin formulation, as confirmed by the observation that no changes in the level of residual C-peptide secretion were observed regardless of the type of insulin that was administered. This indicates that there was no increased stimulation of residual beta cell function in reducing blood glucose levels. Therefore, the metabolic effect observed with buccal spray insulin is the result of a hypoglycemic effect mediated by insulin administered in this form. Although not statistically different, blood glucose levels appeared slightly higher in the buccal spray insulin–treated patients during the test as is also mirrored by the slightly lower insulin levels compared to those obtained with subcutaneous regular insulin. It is likely that this result is in part the consequence of under dosing buccal spray insulin; however, in the 3 patients undergoing continuous glucose monitoring over a 3-day period, appropriate adjustments of puffs were made to optimize the dose of buccal spray insulin dose which resulted in very similar glucose values when compared to subcutaneous regular insulin.

Regarding duration of action, it appears that buccal spray insulin has shorter hypoglycemic action compared to subcutaneous regular insulin, which suggests that it provides less risk of hypoglycemia during chronic use than conventional regular insulin. In patients with type 1 diabetes, the most frequently used therapy in controlling blood glucose levels consists of 3 boluses of insulin (mainly analogues) at meal times with the addition of intermediate insulin in the morning and/or at bedtime. In our study, we did not administer intermediate insulin in the morning of the test because the aim was to measure the hypoglycemic effect of preprandial buccal spray insulin without any interference by intermediate insulin. In clinical practice, however, the addition of intermediate insulin via the subcutaneous route could decrease the amount of buccal spray insulin to be administered at meals, thus limiting the number of puffs required to control blood glucose.

Recently, a different noninvasive route of insulin administration (ie, inhaled) has been evaluated in patients with type 1 diabetes on a basis of a “proof of concept study” [12] or to evaluate the dose response [13]. Although the data are interesting, the fact that a different preparation of long-acting insulin was given in conjunction with the inhaled insulin does not allow one to determine the real contribution of inhaled insulin in controlling blood glucose levels. Like all new insulin delivery systems, long-term studies are required to exclude any adverse effects on the tissue site of absorption [14].

Although our study only included a short-term test, it was able to demonstrate that insulin administered by the buccal route produces target postprandial glucose levels in patients with type 1 diabetes. Therefore, we can postulate that buccal spray insulin could be used to control glucose levels not only in patients with type 2 diabetes as previously reported [6] but also in patients with type 1 diabetes.

Although buccal spray insulin therapy was limited in our study to cover a standard meal and treatment was extended up to just 2 consecutive days, the data are nevertheless interesting because patients were classical type 1 and no intermediate subcutaneous insulin was added during the test to control blood glucose. There is no doubt that insulin administered via this alternative and noninvasive route is a long-awaited hope for patients with diabetes and the present findings represent a *preliminary* significant step in fulfilling this hope. Finally, the buccal spray insulin route should bypass any potentially harmful effects at the pulmonary level because it is not absorbed at this level.

In conclusion, although our results are preliminary and obtained in a small number of patients, the administration of insulin via the buccal route for transmucosal absorption certainly represents a very attractive alternative to subcutaneous insulin injections. However, further studies on larger numbers of patients are required to ascertain safety issues and to determine the effect of buccal spray insulin on metabolic control over a longer period. Also important would be the comparison between buccal spray insulin and short-acting insulin analogues whose time and length of action are more similar to buccal spray insulin than regular insulin.

Acknowledgments

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