Comparative Study of Effectiveness of Multiple-Daily Injections of Insulin Versus Twice-Daily Injections of Biphasic Insulin in Patients with Type 2 Diabetes

Uru NEZU, Akinobu NAKAMURA, Kazutaka AOKI, Mari KIMURA and Yasuo TERAUCHI

Department of Endocrinology and Metabolism, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Received July 18, 2008; Accepted October 31, 2008; Released online November 20, 2008

Correspondence to: Yasuo Terauchi, M.D., Ph.D., Department of Endocrinology and Metabolism, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Abstract. To evaluate the efficacy of a multiple-daily injection regimen and a twice-daily injection regimen using biphasic insulin, we performed an observational study of 56 insulin-naïve patients with type 2 diabetes mellitus who began receiving insulin therapy while they were hospitalized. The subjects were divided into two groups: a multiple-daily injection group (n=33), and a twice-daily injection group (n=23). At baseline, the demographic and clinical characteristics were comparable between the two groups. The HbA1c levels were 10.0±1.6% and 9.5±2.2% (p=0.36), respectively. At 12 weeks, the HbA1c levels decreased equally in the two groups (7.2±1.8% in the multiple-daily injection group and 7.3±1.6%, p=0.80 in the twice-daily injection group). The baseline HbA1c, the duration of diabetes, and the endogenous insulin secretory capacity did not affect the change in HbA1c in either group. These results suggest that twice-daily insulin regimen using biphasic insulin is as effective and beneficial as multiple-daily injection regimen for the treatment in type 2 diabetic patients with very poor glycemic control and that in order to achieve the targeted glycemic goal, insulin therapy should be initiated at an early stage.

Key words: type 2 diabetes mellitus, insulin naïve, multiple-daily injection, twice-daily injection, biphasic insulin

A broad consensus exists regarding the efficacy of stringent glycemic control in the management of diabetes to prevent complications and cardiovascular diseases. The results of the Diabetes Control and Complications Trial (DCCT) conducted in the United States and Canada [1, 2], as well as those of several other European and American studies like the Steno studies [3, 4], the Kroc study [5], and the Oslo studies [6, 7], have indicated that intensive insulin therapy can retard the onset and progression of nephropathy, neuropathy and retinopathy in patients with insulin-dependent diabetes mellitus (IDDM). Furthermore, Ohkubo et al. [8] reported that intensive glycemic control with multiple-daily injections of insulin can delay the onset and the progression of the early stages of diabetic retinopathy, nephropathy and neuropathy in NIDDM patients. Thus, multiple-daily insulin injections are widely accepted as the best treatment option for initiating insulin therapy to achieve sufficient glycemic control.

However, recent evidence suggests that healthcare providers may be slow to act on the need to initiate insulin therapy and that an HbA1c level of 9% or greater is required before healthcare providers will initiate aggressive glucose-lowering therapy [9, 10]. This reluctance may, in part, be due to the unwillingness and/or inability of patients to inject...
insulin. In daily practice, many healthcare providers thus face a dilemma: while multiple-daily insulin injection therapy is preferable for ensuring good glycemic control, it is somewhat inconvenient.

Given these facts, healthcare providers should set a recommendation for initiating insulin therapy that will enable sufficient glycemic control using a regimen comprising fewer injections; such a regimen would be more practical for patients.

Historically, a fixed mixture of human insulin 30/70 or an insulin analog, like insulin lispro mixture 50/50 (50% lispro, 50% insulin lispro protamine suspension, NPL) or biphasic insulin aspart 30/70 (30% aspart, 70% insulin aspart protamine suspension), have been used in twice-daily dosing regimens. These regimens require only two daily injections (before breakfast and supper), allowing patients to experience a more comfortable daily life than with multiple-daily injection regimens.

A few previous reports have compared a twice-daily regimen using biphasic insulin and a multiple-daily insulin injection regimen. Liebl et al. [11] reported that twice-daily injections of premixed insulin analogue were as useful as multiple daily injections in insulin-naïve patients in the United States. Consistent with this finding, Hirao et al. [12] recently reported that twice-daily injections of biphasic insulin aspart were as useful as multiple daily injections in insulin-naïve patients in Japan.

However, whether a twice-daily insulin regimen is equally effective as a multiple-daily injection regimen remains unproved. To obtain conclusive evidence, we performed a retrospective observational study comparing glycemic control using multiple-daily injections of any kind of insulin versus twice-daily injections of biphasic insulin in insulin-naïve patients with type 2 diabetes mellitus.

**Materials and Methods**

We performed a retrospective observational study to analyze clinical data for adult type 2 diabetic patients recruited at Yokohama City University Hospital. The eligible patients comprised 56 insulin-naïve type 2 diabetic patients who began receiving insulin therapy while they were hospitalized at some time between December 2005 and June 2007. Prior to admission, patients were treated with either (1) diet/exercise therapy or (2) one or more oral antidiabetic drugs (OADs) but did not reach the targeted glycemic goal according to ADA guidelines (HbA1c<7.0%).

After admission, patients were initiated with insulin therapy: either by multiple-daily injection regimen which consisted of more than thrice daily injections of any kind of insulin or twice-daily injection regimen which consisted of twice daily injections of premixed biphasic insulin. Multiple-daily injection regimen was selected as the best treatment option to achieve utmost stringent glycemic control. However, because there was no shared protocol regarding the treatment, the regimen was selected by physician’s discretion. In addition, physicians flexibly responded to patients who desired to simplify insulin regimen by decreasing the number of insulin injections. Thus, we divided the patients into two groups based on the insulin regimen on the day of discharge (multiple-daily injection group versus twice-daily injection group).

Self monitoring blood glucose (SMBG) was intensified during hospitalization (sixth per day: before and after every meal). Insulin dose was optimized by the attending physician to achieve target goal of blood glucose (fasting and preprandial glucose <130 mg/dl and postprandial glucose <180 mg/dl), but there was no shared algorithm among the physicians.

All the patients were followed at the outpatient clinic in Yokohama City University Hospital after their discharge, and their HbA1c levels were measured during each visit. We compared the HbA1c levels between the two groups before and after 12 and 24 weeks
of insulin treatment for all the subjects and also for subgroups determined according to their baseline HbA1c levels (< 10% or ≥ 10%).

We further evaluated the impact of several factors that might have affected the efficacies of the insulin regimens. First, we divided all the subjects into two subgroups with respect to the duration of their diabetes (greater or less than the median value of 14.4 years) and evaluated the HbA1c levels of the two subgroups. Secondly, we divided all the subjects into two subgroups with respect to their C-peptide index (C-peptide index > 1.2 and ≤ 0.8), which was defined by the following equation: fasting C-peptide (ng/mL) divided by fasting glucose (mg/dL) × 100, representing endogenous basal insulin secretory capacity [13]. We then compared the HbA1c levels between the two subgroups.

Statistical analyses were conducted using SPSS software, version 16J for Windows. Comparisons between the multiple-daily injection group and the twice-daily injection group were performed using a Wilcoxon two-sample test. All data were expressed as the mean ± S.D. A p-value < 0.05 was considered statistically significant.

Results
After hospitalization, total of 67 insulin naïve patients began insulin therapy: multiple-daily injection regimen (n=50) and twice-daily injection regimen (n=17). Among those, patients with poor glycemic control tended to be allocated to multiple-daily injection regimen. However, before the day of discharge, 9 patients of multiple-daily injection regimen switched to the twice-daily injection regimen on their request to simplify the regimen, while all patients in twice-daily injection regimen continued the allocated regimen. Thus, 41 and 26 patients were incorporated to a multiple-daily injection group and a twice-daily injection group, respectively. During follow-up period, 8 patients in a multiple-daily injection group and 3 patients in a twice-daily injection group discontinued the allocated insulin regimen or were lost to follow up, thus 33 and 23 patients were finally analyzed (Fig. 1).

The demographic and clinical characteristics at baseline are outlined in Table 1. Patients in both groups were similar with respect to age, BMI, the duration of diabetes, and HbA1c (Table 1). The difference in sex distribution between the two groups (higher proportion of men in the multiple-daily injection group) was not statistically significant. Although body weight and BMI was comparable between the two groups at baseline, changes during the follow-up period were not analyzed because of the lack of measurement of body weight in most cases.

As for the treatments prior to insulin induction, 7 of the 33 patients (21%) in the multiple-daily injection group were on diet and exercise therapy and the remaining 26 patients (79%) were being treated with OADs. On the other hand, 2 of the 23 patients (9%) in the twice-daily injection group were on diet and exercise and the remaining 21 patients (91%) were being treated with OADs. Prior treatment and insulin regimen did not significantly differ (p=0.28).

The regimens in the multiple-daily injection group consisted of (1) thrice-daily injections of insulin aspart (n=7), (2) thrice-daily injections of insulin aspart with NPH insulin (n=19), (3) thrice-daily injections of human insulin with NPH insulin (n=2), (4) twice-daily injections of human insulin with premixed human insulin 30/70 (n=1), (5) thrice-daily injections of premixed insulin aspart 30/70 (n=2), or (6) thrice-daily injections of premixed insulin lispro 50/50 (n=2). The regimens in the twice-daily injections group consisted of twice-daily injections of (1) premixed insulin aspart 30/70 (n=17), (2) premixed insulin lispro 50/50 (n=1), or (3) premixed human insulin 30/70 (n=5).

At baseline, HbA1c were 10.0±1.6% and 9.5±2.2% in the multiple-daily injection group and the twice-daily injection group, respectively (p=0.16) (Fig. 2). During follow-up period, HbA1c decreased equally between the two groups. (7.2±1.8% and
7.3±1.6%, p=0.45 at 12 weeks and 7.4±2.0% and 7.2±1.8%, p=0.83 at 24 weeks, respectively), suggesting that twice-daily injection regimen may improve glycemic control as effectively as multiple-daily injection regimen.

Interestingly, the total daily insulin doses at baseline were significantly greater in the multiple-daily injection regimen than in the twice-daily injection regimen (24.6±11.1 units vs. 17.0±5.9 units, p=0.005). Insulin doses were slightly increased in the twice-daily injection regimen but was still greater in the multiple-daily injection regimen (24.1±11.6 units vs. 20.1±6.0 units, p=0.099 at 24 weeks).

We then compared HbA1c between subgroups according to baseline HbA1c levels (<10% or ≥10%). The HbA1c reached at 12 and 24 weeks were better in the lower HbA1c subgroups. In the lower HbA1c group (<10%), the HbA1c levels were 8.5±0.8 and 8.3±0.8% (p=0.72) at baseline, 6.5±0.9% and 6.9±1.0% (p=0.25) at 12 weeks, and 6.5±1.5% and 6.4±1.0% (p=0.82) at 24 weeks in the multiple-daily injection group and the twice-daily injection group, respectively. In comparison in the higher HbA1c group (≥10%), the HbA1c levels were 11.2±0.9 and 11.9±2.0% (p=0.28) at baseline, 7.9±2.1% and 8.1±2.2% (p=0.63) at 12 weeks, and 8.2±2.2% and 8.7±2.6% (p=0.64) at 24 weeks in the multiple-daily injection group and the twice-daily injection group, respectively.

Among the patients whose therapy prior to insulin induction consisted of only diet and exercise (early induction of insulin subgroup), the mean HbA1c level changed from 10.6±1.9% to 6.6±1.3% in those who received multiple-daily injections (n=7) and from 12.2±1.3% to 7.5±0.2% in those who received twice-daily injections (n=2) over the course of a 12-week treatment period (Fig. 3). No difference in this parameter was observed between the two treatment groups (mean reductions: -4.0±2.3% and -4.7±1.6%, respectively; p=0.89). Among the patients who had taken OADs prior to insulin induction, the mean HbA1c level changed from 9.8±1.5% to 7.4±1.9% in those who received multiple-daily injections (n=26) and from 9.5±2.2% to 7.3±1.7% in those who received twice-daily injections (n=21) over the course of the 12-week treatment period. Again, no difference between the two treatment groups was observed (mean reductions: -2.3±2.0% and -2.1±2.4%, respectively; p=0.52).

In the multiple-daily injection group, the duration of diabetes among the patients with early insulin induction was significantly shorter than among those who had taken OADs (6.3±10.9 vs. 16.6±10.2 years, p=0.011). In the twice-daily injection group, no statistical difference in the duration of diabetes was seen between the two subgroups (17.5±3.5 vs. 14.0±9.9 years). Comparing these two prior-treatment subgroups, however, the decrease in the HbA1c level among the patients in the early insulin induction group was greater than that observed among the patients who had previously taken OADs, although the difference was not statistically significant (Fig. 3).

We next evaluated the impact of the duration of diabetes on the change in the HbA1c level. In the long duration group (duration > 14.4 years), the HbA1c levels were 9.5±1.5 and 9.6±2.0% (p=0.31) at baseline, 7.3±2.1% and 7.9±1.9% (p=0.98) at 12 weeks, and 7.6±2.3% and 7.7±2.1% (p=0.53) at 24 weeks in the multiple-daily injection group and the twice-daily injection group, respectively (Fig. 4). In the short duration group (duration < 14.4 years), the HbA1c levels were 10.3±1.6% and 9.9±2.6% (p=0.56) at baseline, 7.2±1.6% and 6.9±1.3% (p=0.63) at 12 weeks, and 7.2±1.9% and 6.6±1.6% (p=0.36) at 24 weeks in the multiple-daily injection group and the twice-daily injection group, respectively.

We then evaluated the impact of endogenous insulin secretion on the change in HbA1c. In the group whose C-peptide index was > 1.2, the HbA1c levels were 9.3±1.3% and 9.1±1.3% (p=0.79) at baseline, 6.7±1.3% and 7.0±1.6% (p=0.63) at 12 weeks, and 6.8±1.8% and 6.9±1.9% (p=0.92) at 24 weeks in the multiple-daily injection group and the twice-daily injection group, respectively (Fig. 4). In the group whose C-peptide index ≤
0.8, the HbA1c levels were 10.3±1.3 and 11.6±3.1 (p=0.26) at baseline, 8.2±2.4% and 7.4±0.3% (p=0.39) at 12 weeks, and 9.0±2.4% and 7.1±0.8% (p=0.16) at 24 weeks in the multiple-daily injection group and the twice-daily injection group, respectively.

**Discussion**

The most important finding in this study was that the mean HbA1c level decreased by approximately 2.5% after 12 and 24 weeks of receiving either an intensified therapy of multiple-daily injections or a conventional therapy of twice-daily injections of biphasic insulin. This result was comparable to those obtained in previous reports, suggesting that glycemic control using a twice-daily injection regimen may be as effective as a multiple-daily injection regimen in insulin-naïve patients [11, 12]. Furthermore, the twice-daily injection regimen achieved an equal glycemic control to that of the multiple-daily injection regimen even though the insulin dose was significantly lower. This point is clinically important because it may prevent unwanted weight gain caused by the long-term use of insulin injections, a phenomenon that was observed in the DCCT trial [14].

According to subgroup analysis according to the baseline HbA1c, the HbA1c reached was better in the lower HbA1c subgroups, indicating that early intervention of insulin therapy is important. In addition, subgroup analysis according to the treatment prior to insulin induction showed compatible data; HbA1c level after 12 weeks was reduced to a greater extent among patients who had not previously taken OADs (early insulin induction subgroup), compared with those who had taken these drugs, regardless of the insulin regimen. The early insulin induction subgroup had a shorter duration of diabetes, though no statistical difference in the twice-daily injection group was seen.

A similar result was previously reported by Hirao et al. [12]. In that paper, the authors proposed that the shorter duration of diabetes might have contributed to a reserve of beta-cell function, since the plasma glucose level gradually rises with the progressive deterioration of beta-cell function during the natural course of diabetes [17, 18].

Next, C-peptide index, which represents the endogenous basal insulin secretory capacity and thus beta-cell function, was comparable between the two subgroups. So, we further evaluated the prandial insulin secretory capacity by calculating the prandial C-peptide index (defined as prandial C-peptide (ng/mL) divided by prandial glucose (mg/dL) ×100) in the two subgroups; however, no statistical difference was seen (1.7±1.2 and 1.5±1.0, p=0.853). Nevertheless, these results are not conclusive because of the small sample size.

Finally, to assess the impact of the duration of diabetes and beta-cell function on glycemic control, we conducted subgroup analyses according to the duration of diabetes and the C-peptide index. As a result, glycemic control was equally improved, irrespective of these factors, in both insulin regimens, indicating that a twice-daily injection of biphasic insulin might be sufficient to compensate for the deficit in endogenous insulin secretory capacity.

So far in our study, both insulin regimen equally improved glycemic control. However, this finding cannot be still applied to all cases, since the HbA1c reached was quite insufficient considering today’s recommendation by the AACE [15] and the ADA [16] (HbA1c<6.5% and <7.0%, respectively). To obtain more stringent glycemic control without increasing the risk of hypoglycemia, multiple-daily injection may be superior to twice-daily injection regimen.

Several limitations should be recognized because we adopted a retrospective experimental design. First, because treatment selection (multiple-daily injection versus twice-daily injection) throughout the study period were deferred to negotiation between physicians and patients, it is impossible to exclude potential biases in the treatment
selection. Thus, subjects’ characteristics might be unequally distributed between the groups. Of note, 9 patients switched from multiple-daily injection to twice-daily injection. Thus, more patients with poor adherence to diet therapy might have assembled into the twice-daily injection group. One method to verify their adherence would be to compare their BMI before and after the intervention, which was missing from our data, as we already described above. Thus, our study lacks several outcomes of great interest. This is the second limitation. Finally, the number of subjects in our experimental design was smaller than that in many other studies [11, 12].

Therefore, our findings only suggest that the twice-daily injection of biphasic insulin is useful for a wide variety of patients irrespective of their diabetes history and endogenous insulin secretion. Thus, our survey is more relevant as an observational study than as a hypothesis-testing one. Larger-scale prospective studies should be performed in the future.

In summary, the present results suggest that, in addition to intensive therapy with multiple insulin injections, convenience-oriented insulin therapy using biphasic insulin injections is a sufficiently useful option for the treatment in type 2 diabetic patients with very poor glycemic control.

Acknowledgements

This work was supported in part by the Yokohama City University Center of Excellence Program and a grant for the 2007 Strategic Research Project (K19004) of Yokohama City University.

References


Table 1. Baseline characteristics of patients

<table>
<thead>
<tr>
<th></th>
<th>Multiple-daily injection group</th>
<th>Twice-daily injection group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n</td>
<td>33</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Prior therapy (Diet/exercise only /Oral antidiabetic agents), n</td>
<td>7/26</td>
<td>2/21</td>
<td>0.28</td>
</tr>
<tr>
<td>Oral antidiabetic agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SU</td>
<td>65.3%</td>
<td>71.4%</td>
<td></td>
</tr>
<tr>
<td>TZD</td>
<td>30.8%</td>
<td>10.7%</td>
<td></td>
</tr>
<tr>
<td>BG</td>
<td>11.5%</td>
<td>17.9%</td>
<td></td>
</tr>
<tr>
<td>Glinide</td>
<td>0.0%</td>
<td>10.7%</td>
<td></td>
</tr>
<tr>
<td>α-GI</td>
<td>23.1%</td>
<td>46.4%</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F), n</td>
<td>22/11</td>
<td>16/6</td>
<td>0.43</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.0±11.1</td>
<td>64.3±9.6</td>
<td>0.46</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>14.4±10.9</td>
<td>15.4±9.3</td>
<td>0.70</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10±1.6</td>
<td>9.5±2.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>61.8±13.3</td>
<td>59.0±11.3</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2±4.1</td>
<td>22.2±3.0</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Data are presented as means±S.D., number of patients
67 eligible participants

**Insulin regimen at induction**

- Multiple-daily injection (n=50)
  - 41 received multiple-daily injection on the day of discharge
    - 4 did not receive allocated intervention:
      - Death by cancer (n=1)
      - Withdrawed from insulin therapy (n=1)
      - Switched to twice-daily injection (n=2)
  - Follow up at:
    - Week 12: 33
    - Week 24: 33
  - 33 analysed
  - 8 excluded from analysis:
    - Discontinued allocated intervention (n=4)
    - Lost to follow up (n=4)

- Twice-daily injection (n=17)
  - 26 received twice-daily insulin regimen on the day of discharge
    - 3 did not receive allocated intervention:
      - Switched to multiple-daily injection (n=2)
      - Switched to once-daily injection (n=1)
  - Follow up at:
    - Week 12: 23
    - Week 24: 23
  - 23 analysed
  - 3 excluded from analysis:
    - Discontinued allocated intervention (n=3)

**Fig. 1.** Flow diagram of the study. The diagram included the number of patients followed up at different times during the study period.

**Fig. 2.** Changes in HbA1c levels in patients receiving multiple daily injections (square, n=33) or twice-daily injections (circle, n=23). At 12 weeks, the mean HbA1c values in the two groups had changed from 10.0 ±1.6% to 7.2 ±1.8% and from 9.5±2.2% to 7.3±1.6%, respectively. The mean HbA1c levels had decreased by approximately 2.5% at 12 weeks, compared with the baseline, and were maintained at this level for at least 24 weeks in both groups. No statistical differences between the two groups were seen at baseline, or at 12 or 24 weeks. Data are shown as the mean ± S.D.
Fig. 3. Reduction of HbA1c levels at 12 weeks in the two treatment groups according to treatment prior to insulin induction. Patients who underwent early insulin induction exhibited a greater reduction in their HbA1c levels than those who had taken OADS in either treatment group, irrespective of the insulin regimen.

Fig. 4. Impact of duration of diabetes on the reduction in HbA1c levels at 12 weeks. Long duration group: multiple daily injections (n=13) and twice-daily injections (n=11); short duration group: multiple daily injections (n=19) and twice-daily injections (n=12). No difference between the insulin regimens was seen. Data are shown as the mean ± S.D.
Fig. 5. Impact of endogenous insulin secretion on the reduction in HbA1c levels at 12 weeks. C-peptide index $\geq$ 1.2: multiple daily injections (n=11) and twice-daily injections (n=8), C-peptide index $\leq$ 0.8: multiple daily injections (n=10) and twice-daily injections (n=5). No difference between the insulin regimens was seen. Data are shown as the mean $\pm$ S.D.