ABSTRACT

Objective: To compare how the rapid-acting insulin analogues (RAIAs) aspart, lispro, and glulisine perform in continuous subcutaneous insulin infusion (CSII) therapy regarding (1) pharmacokinetic properties, (2) chemical and physical stability, and (3) pump compatibility.

Methods: PubMed was searched for articles pertaining to the use of RAIAs in CSII, without a restriction on the time period.

Results: These RAIAs have pharmacokinetic profiles that more closely mimic endogenous insulin in comparison with regular human insulin and tend to produce less hypoglycemia. Among these RAIAs, the rates of absorption and clinical efficacy in terms of glycemic control were similar. Although glulisine showed a faster onset of action in some studies with aspart and lispro, this advantage lasted only for a maximum of 1 hour, after which results were similar for glulisine and aspart or lispro. Each RAIA is created by making minor amino acid substitutions to the regular human insulin molecule and adding a stabilizer to help prevent fibrillation. A series of chemical and covalent changes affecting the primary structure of an insulin preparation, however, may cause decomposition during storage, handling, and use, diminishing the potency of the insulin molecule while contained in an insulin pump. Precipitation, fibrillation, and occlusion may ensue, undermining compatibility for CSII pump use. Aspart has demonstrated the greatest chemical and physical stability in the insulin pump, with the lowest rates of overall occlusion in comparison with lispro and glulisine (aspart 9.2%, lispro 15.7%, and glulisine 40.9%; P<.01).

Conclusion: Aspart is the most compatible of the 3 RAIAs for pump use. (Endocr Pract. 2011;17:271-280)

INTRODUCTION

Nearly 35 years after its introduction, continuous subcutaneous insulin infusion (CSII) continues to grow in use worldwide among patients with type 1 or type 2 diabetes. Studies show that, in comparison with multiple-dose injection therapy, CSII therapy requires a lower insulin dosage, yields similar or superior outcomes in terms of mean blood glucose concentrations and hemoglobin A1c (A1C) levels, and is associated with lower rates of hypoglycemia, greater treatment satisfaction, and a similar incidence of diabetic ketoacidosis (1-11).

The rapid-acting insulin analogues (RAIAs) aspart, lispro, and glulisine have all been approved by the US Food and Drug Administration for use in CSII therapy in adults, with aspart being approved for CSII therapy in pediatric patients as well. Although there are only minor differences
in the molecular structure between these analogues and regular human insulin (RHI), the pharmacokinetic profiles of the RAIAs more closely mimic that of endogenous insulin in comparison with RHI (7,8,12-28). Several studies comparing these analogues with RHI in CSII therapy reflect the benefit of this improved pharmacokinetic profile, resulting in better postprandial glycemic control and a trend toward less hypoglycemia (12-15,17,19,20,24-28).

The efficacy of any insulin preparation depends on how stable it is in the insulin pump. The stability may be compromised by various circumstances, including an extended increase in temperature, change of pH, agitation, or contact with hydrophobic surfaces. If the stability of an insulin preparation is compromised, structural changes may occur in the insulin molecule. In this review, the performance of insulin aspart, lispro, and glulisine in CSII will be compared regarding (1) pharmacokinetic properties, (2) chemical and physical stability, and (3) pump compatibility.

PHARMACOKINETIC PROPERTIES OF RAIAs

The RAIAs were designed to provide enhanced pharmacokinetic properties that ensure a more physiologic insulin profile in comparison with RHI. RHI is a solution of zinc-insulin hexamers, in which zinc has been added to increase chemical stability by promoting hexamer association (29,30). The consequent tendency of RHI to self-associate into hexamers (and dimers) slows the rate of absorption after subcutaneous delivery because these relatively large complexes have difficulty passing through capillary membranes (31). Therefore, a lag phase occurs while self-associated RHI hexamers gradually dissociate into monomers that are more readily absorbed into the bloodstream (32). People with diabetes are not always able to adhere to the 30-minute recommended meal-related injection schedule for RHI, often injecting within <30 minutes before a meal (33). As a result, the injected RHI reaches the bloodstream after blood glucose levels are already elevated and postprandial hyperglycemia has occurred. In addition, the delayed entry of insulin into the circulation results in delayed insulin action, which can increase the risk of hypoglycemia (31,32,34).

Insulin aspart, lispro, and glulisine were produced by making minimal amino acid substitutions to the RHI molecule at those sites involved in self-association, allowing the analogues to dissociate more easily than RHI and remain monomeric even at the high concentrations necessary to achieve the required potency (16,32,35-38). This rapid dissociation results in a beneficial pharmacokinetic profile, including rapid increases in plasma insulin concentrations; thus, the 30-minute preprandial administration recommended in association with RHI is obviated (31,32,36,39).

Specifically, the RAIAs were created by substituting various residues on the B chain of human insulin (Fig. 1) (40-42). Phosphate buffers were added to RHI in the 1980s and, more recently, to insulin analogues to enhance physical stability, particularly to prevent fibril formation. The addition of these buffers has helped decrease, but not eliminate, catheter occlusion (43-45). Similarly, stabilizers are added to insulin analogues to prevent fibrillation (46). Zinc is added to aspart and lispro and acts as a hexamer stabilizer, promoting insulin self-association and monomer formation to counteract fibrillation (40,41). Although no zinc is added to glulisine, the surfactant polysorbate 20 is added to prevent interactions between insulin and hydrophobic surfaces in the pump (42,46). The outcome of these molecular modifications has been examined in studies that compared the 3 analogues in terms of rate of absorption and clinical efficacy.

Rate of Absorption

Comparative studies highlight the fast absorption and peaked profile of all RAIAs, with each having a faster rate of absorption than RHI after subcutaneous injection, as shown in Figure 2 (12-14). In comparisons of glulisine with aspart and lispro (19,47-49), some evidence indicated that the absorption of glulisine begins sooner after injection than that of the comparators, based on the criterion of time to 10% of the maximal glucose infusion rate (GIR-\textsuperscript{10%}[\text{min}]). The actual $t_{\text{max}}$, however, occurred earlier with aspart than glulisine. A crossover study by Arnolds et al (47) demonstrated that glulisine had a faster onset of action than aspart in healthy subjects after subcutaneous injections of 0.2 U/kg of glulisine or 0.2 U/kg of aspart under euglycemic glucose-clamp conditions occurring on 2 visits separated by a washout period of 5 to 28 days. Glulisine, in comparison with aspart, produced significantly greater glucose-lowering activity during the first 30 minutes (area under the curve [AUC]-GIR\textsubscript{0-30 min} [mg/kg]), but not over the first hour (Table 1). Interestingly, aspart had a significantly faster time to total maximal GIR (GIR-\textsubscript{max} [\text{min}]). Moreover, glulisine and aspart were similar in terms of time to maximal observed insulin concentration and total metabolic activity (Table 1) (47).

These results are similar to a previous study by Heise et al (48), in which 80 adults without diabetes experienced a greater glucose-lowering effect with glulisine versus lispro only in the first hour. As shown in Table 1, glulisine was significantly more rapid than lispro in achieving 10% of GIR-AUC\textsubscript{0-10 h} (GIR-\textsubscript{10%} [\text{min}]). Total serum insulin and glucose control, however, were the same for both analogues over 10 hours (48).

Significantly faster absorption rates with glulisine versus lispro were also documented in a post hoc analysis of an open-label, crossover study of 18 obese subjects with type 2 diabetes (49). Injections of lispro or glulisine were given within 2 minutes of each of three 500-kcal standard test meals spaced 4 hours apart on 2 separate 12-hour visits. In the original trial, the mean preprandial-subtracted glucose AUCs for the glulisine and lispro treatments were similar
(218.94 versus 224.32 mmol/L/min). After glulisine treatment, however, the mean of the 3 maximal preprandial-subtracted plasma glucose concentrations was approximately 12% lower versus lispro (3.55 versus 4.06 mmol/L; \( P < .01 \)) for the total study period (12 hours). Additionally, a post hoc analysis showed that faster absorption in the first 30-minute postmeal period (estimated difference 0.48 \( \mu \)U/min; \( P < .0001 \)) occurred in subjects receiving glulisine versus lispro (49). The use of 500-kcal test meals instead of “real-life” meals, however, does not reflect the actual typical eating habits of patients with type 2 diabetes. Thus, even though the aforementioned studies show a faster onset of action for glulisine than for lispro within the first 60 minutes, the clinical relevance is unknown.

Additionally, despite the very rapid absorption of RAIA s in general, several studies of the pharmacodynamic effects of RAIA s have shown that optimal limitation of postprandial plasma glucose excursions is achieved when a subcutaneous bolus is administered about 20 minutes before a meal (50,51). Thus, CSII may be most effective in this respect if the meal-related infusion begins slightly in advance of eating.

**Fig. 1.** Primary molecular structures of the rapid-acting analogues, showing amino acid substitutions. A, Insulin aspart (40). B, Insulin lispro (41). C, Insulin glulisine (42).
Fig. 2. Pharmacodynamic (left vertical panel) and pharmacokinetic (right vertical panel) curves of the 3 rapid-acting insulin analogues in comparison with regular human insulin (RHI). A and B, Insulin aspart. Serial mean serum glucose levels (A) and serial mean serum free insulin concentrations (B) determined up to 6 hours after a single dose of insulin aspart or RHI was injected immediately before consumption of a meal in 22 patients with type 1 diabetes. [A and B from Lindholm et al (14). Diabetes Care. 1999;22:801-805. Copyright 1999, American Diabetes Association. Reprinted with permission from the American Diabetes Association.] C and D, Insulin lispro. Blood glucose levels (C) and serum RHI and insulin lispro levels (D) after subcutaneous injection of RHI or insulin lispro (0.2 U/kg) immediately before consumption of a high-carbohydrate meal in 10 patients with type 1 diabetes. [C and D from Heinemann et al (12). Diabet Med. 1996;13:625-629. Reprinted with permission from John Wiley and Sons.] E and F, Insulin glulisine. Serial mean blood glucose levels (E) determined up to 6 hours after a single dose of insulin glulisine was given immediately before consumption of a meal versus RHI given immediately before a meal in patients with type 1 diabetes who received a dose of 0.15 U/kg. [E and F from Rave et al (13). Diabetes Care. 2006;29:1812-1817. Copyright 2006, American Diabetes Association. Reprinted with permission from the American Diabetes Association.]
Clinical Efficacy Comparisons

One study noted no distinguishable differences between glulisine and aspart in terms of total serum insulin and glucose control in CSII (52). Hoogma and Schumicki (52) compared CSII bolus glulisine and aspart immediately before meals (in addition to CSII basal insulin) in 59 subjects with type 1 diabetes. After 12 weeks, A1C values and daily insulin doses were similar for each analogue. The mean change in A1C from baseline to end point was +0.2% for glulisine (from 6.8% to 7.0%) and +0.1% for aspart (from 7.1% to 7.2%). Glulisine and aspart also had similar mean total daily doses at the conclusion of the study (43.3 versus 44.4 IU, respectively). A slight increase in mean daily bolus dose occurred with glulisine (+1.0 IU) and aspart (+1.5 IU) (52).

Additionally, a multinational study involving >600 patients with type 1 diabetes being treated by subcutaneous injection indicated no clinical difference in A1C levels or incidence of hypoglycemia between glulisine and lispro therapy (53). After 26 weeks of treatment, the adjusted mean change in A1C from baseline was -0.14% in both groups, and the rates of symptomatic, severe, and nocturnal hypoglycemia were similar between treatment groups (3.64, 0.03, and 0.55 events/patient-month, respectively, for glulisine versus 3.48, 0.02, and 0.53, respectively, for lispro). The total daily insulin dose, however, decreased in the glulisine group (-0.86 IU) but increased in the lispro group (1.01 IU) \( (P = .0123) \).

Although some CSII, subcutaneous injection, and glucose clamp studies indicate equivalent postprandial glucose control for the insulin analogues (39,48,50,54), others indicate meaningful differences among aspart, lispro, and glulisine (15,48,49,55,56). On the basis of the limited amount of data gathered to date, however, it may be best to conclude that there are essentially no differences in glycemic control among the RAIAs used in CSII.

**CHEMICAL AND PHYSICAL STABILITY AND PUMP COMPATIBILITY OF RAIAs**

Before RHIs can exert any blood glucose-lowering effects, a series of chemical and covalent changes affecting its primary structure can cause deterioration of the molecule during storage, handling, and use, rendering the molecule less potent (46). Such decomposition may result in precipitation, fibrillation, and occlusion, undermining
compatibility for CSII pump use (35,46). Several studies have compared the RAIAs to each other and to buffered RHI in terms of chemical and physical stability and pump compatibility.

Chemical and Physical Stability

Various research reports suggest that the material oculding catheters in insulin pumps may be fibrin deposition, insulin fibrils, or isoelectric precipitation of insulin due to a decline in pH caused by carbon dioxide diffusion through the catheter (43,57,58). Isoelectric precipitation can cause pharmacokinetic changes in insulin products, leading to occlusion. In an acidic environment, insulin will precipitate; the point at which precipitation commences depends on the isoelectric point of the individual insulin preparation (43).

Poulsen et al (43) compared the pH level at which aspart, lispro, and buffered RHI underwent isoelectric precipitation by lowering environmental pH for each insulin (by means of exposure to carbon dioxide or by adding diluted hydrochloric acid). Aspart demonstrated the highest resistance to isoelectric precipitation, with 10% and 90% of precipitation occurring at lower pH (5.90 and 5.67) than for buffered RHI (6.18 and 5.95) and lispro (6.41 and 6.30), respectively (43). The tendency of aspart to remain stable even if buffer pH decreased was demonstrated again in a later study that compared aspart with glulisine with use of reverse-phase high-performance liquid chromatography, while lowering pH by adding diluted hydrochloric acid (59). The pH at which 50% precipitation occurred was again lower for aspart than for glulisine (pH 5.86 versus 6.64, respectively) (59).

Senstius et al (60) investigated the in vitro stability of aspart. Aspart was stored in a Medtronic MiniMed 508 pump (Medtronic MiniMed, Inc., Northridge, California) at 37°±2°C for up to 7 days. For simulation of real-life use, the pumps were set on a vibrating platform (30 oscillations/min, 2-cm amplitude displacement) for 24 hours a day. The insulin remaining in the pump reservoir was tested on days 3, 4, and 7 and compared with control samples (60). Aspart exhibited no significant reduction in potency, remaining within baseline ±0.8 U/mL during the study. Additionally, no evidence was found of precipitation or fibrillation, indicating no problems with physical stability (60).

Another in vitro study by Senstius et al (61) that compared the stability of aspart and glulisine found that glulisine had reduced stability and tended to form insoluble complexes (fibrils) that could occlude the catheter. The 10-day study evaluated each insulin analogue for physical stability against fibril formation and chemical stability against formation of high-molecular-weight proteins (HMWPs). Aspart- and glulisine-containing plastic reservoirs were positioned in MiniMed 508 insulin pumps and agitated continuously. Insulin samples were then tested for generation of HMWPs and content of insulin. By the 10th day, the physical stability and chemical stability of the glulisine samples were substantially reduced in comparison with aspart. Stability in needle-end samples for glulisine had decreased to 20% of the baseline sample, whereas the aspart sample had increased to 230%. Likewise, stability in the reservoir sample for glulisine had decreased to 70% of baseline in comparison with a 170% increase from baseline for the aspart sample. By day 10, glulisine contained 2 times the amount of biologically inactive HMWPs in comparison with aspart (0.8% versus 0.4%, respectively) (61).

Similarly, the in vitro stability of lispro was examined in 3 CSII pumps (MiniMed 507c, H-TRONplus, and D-TRON CSII; Disetronic Medical Systems, Inc., St. Paul, Minnesota, for the last 2 models) and subjected to high temperatures (37°C) and vibrations (100 strokes/min) for 1 week (62). The pumps were programmed for simulated basal-bolus administration (continuous infusion of 0.8 U/h and 3 daily bolus doses of 6 U). The infused material was collected every 24 hours throughout the week and assessed for potency, purity, and HMWPs. The potency for each pump was within 95% to 105% of label claims each day, with no indication of absorption or denaturation of lispro provoked by pump surfaces. The increase in HMWPs and other degradation products was considerably lower than the specification limits of 1.50%. Furthermore, the infusion lines were clear of precipitates, and no occlusion alarms occurred (62).

Pump Compatibility

How an insulin analogue reacts to storage in an insulin pump and how the pump user reacts to using that insulin analogue in the pump define the “pump compatibility” of an insulin analogue. Gauges for determining insulin pump compatibility are the occurrence of occlusion and treatment satisfaction. Recently, the recommended duration of time that aspart may be kept in the insulin pump reservoir was changed from a maximum of 48 hours to up to 6 days (40). In contrast, prescribing information for lispro and glulisine recommends that unused insulin in the insulin pump reservoir be discarded within 48 hours or less (41,42). In addition, of the 3 insulin analogues, only aspart has been approved for use in CSII in pediatric patients (40-42).

Occurrence of Occlusion

Several studies have compared the occurrence of catheter occlusion between RHI preparations and RAIs or among RAIs (10,15,21,39,43,47,52,53,63-67). For example, some crystal formation occurred with both aspart and buffered RHI in a 7-week CSII study of 19 patients with type 1 diabetes (64). Significantly less crystallization occurred with aspart versus buffered RHI in the reservoir and distal tubing, respectively (64). In another study that compared CSII aspart, buffered RHI, and lispro in patients with type 1 diabetes, most study subjects reported ≤1 clog
or blockage every 4 weeks during the trial for aspart, RHI, and lispro (76%, 83%, and 75%, respectively) (15).

Kerr et al (63) found fewer occlusions with aspart in comparison with lispro or glulisine in an in vitro study on rates of early and late occlusion in standard CSII catheters. Each analogue was tested in 8 insulin pumps, which were programmed to receive 0.1 IU/h of basal doses and 2 IU 3 times daily of bolus doses. Each pump was incubated within 32°C to 36°C to mimic conditions of normal use. The study was conducted for 9 independent 5-day periods. No occlusions occurred within the first 48 hours for any analogue. Within normal working temperatures, aspart was the least likely to occlude, with the lowest probability of overall occlusion at 9.2%, in comparison with glulisine that had the highest risk of occlusion at 40.9% and lispro at 15.7% (Fig. 3). During the third run on the second study day, the incubator temperature was raised in error from 34°C to 40°C for all 3 insulins, returning to normal range on the fourth day. Even at this higher temperature, aspart was again the least likely to occlude, with 5 of 8 pumps occluding versus 6 of 8 with lispro and 7 of 8 with glulisine (63). For all test runs, the difference in occlusion risk among the insulin analogues was significant (P = .0005). The significance level with omission of the third run was P = .0009 (63).

Certain characteristics of glulisine in comparison with other insulin analogues may explain the high occlusion rate found in this study. One possible explanation may be the zinc-free formulation of glulisine. The surfactant polysorbate 20 may not provide as much protection against fibrillation as does the hexamer stabilizer zinc. Moreover, the recommended storage temperature range for glulisine is lower than those for the other 2 insulin analogues: according to their separate labels, glulisine can be stored up to 28 days in temperatures <25°C, whereas aspart and lispro can be stored for the same amount of time, but at temperatures up to 30°C (40-42).

Although the stability of lispro was initially established in a study that used reverse-phase and size-exclusion high-performance liquid chromatography (67), results of later studies and case reports demonstrate greater precipitation and occlusion with lispro in comparison with aspart (65) and repeated cannula occlusion and hyperglycemia with lispro in comparison with RHI (68). For example, 2 case studies reported precipitation in the infusion catheters with lispro (65). In case 1, a 42-year-old woman with type 1 diabetes was switched from buffered RHI to lispro, with use of the MiniMed 507C pump in both circumstances (65). Within 40 hours after changing treatment, the catheter occluded with insulin precipitate, as confirmed by radioimmunoassay (Fig. 4A). After her treatment was changed back to the RHI, the occlusions did not recur. The treatment was later adjusted to insulin aspart; after 5 months of treatment, no catheter occlusions had occurred (65).

Similarly, in case 2, insulin precipitate clogged the infusion catheter after a 31-year-old woman with type 1 diabetes, using a Disetronic H-TRON V-100 pump, had her treatment switched from buffered RHI to lispro (Fig. 4B) (65). The precipitate was confirmed to be insulin when the outer wall of the Sof-Set catheter (Medtronic MiniMed) was removed and stained with dithizone (diphenylthiocarbazone); a white precipitate was revealed. Additionally, the woman had experienced unexpected fluctuations in glucose concentrations while receiving lispro (65). Explanations for such catheter occlusions with lispro are unknown, but such episodes may be attributable to the higher isoelectric point of lispro in comparison with RHI.

The 3 RAIAs demonstrate improved stability over RHI in terms of the occurrence of catheter occlusion. As shown in both in vitro and in vivo studies, of the 3 analogues, aspart is the least likely to occlude or precipitate in the insulin pump (43,59-65).

Treatment Satisfaction With CSII

A MEDLINE search of studies on treatment satisfaction with RAIAs in CSII yielded 1 publication that compared treatment satisfaction with the use of aspart versus lispro in CSII (69). No such studies were found involving glulisine. In the 16-week study by Wittlin et al (69), 439 patients with type 1 or type 2 diabetes previously given CSII with lispro (≥6 months) compared treatment satisfaction with use of lispro versus aspart in CSII (69). The study subjects continued with lispro treatment for 4 weeks before switching to 12 weeks of aspart therapy. Immediately after each treatment period, study subjects completed the Diabetes Treatment Satisfaction Questionnaire and the Insulin Treatment Satisfaction Questionnaire (ITSQ). The overall score for the Diabetes Treatment Satisfaction Questionnaire was similar for lispro and aspart treatments.
(88.2 versus 87.5, respectively); the average overall ITSQ score, however, significantly improved with aspart (82.9) versus lispro (81.2), with a treatment difference of 1.7 ($P = .001$), implying that aspart had a positive effect on treatment satisfaction. Aspart was found noninferior to lispro in all 6 scales evaluated in the ITSQ: ease-convenience, interference burden, lifestyle, hypoglycemic control, glycemic control, and delivery system. In addition, the mean ITSQ score for aspart was significantly higher than that for lispro (1.5 ± 1.5 points for aspart versus 7.1 ± 3.6 points for lispro; $P < .005$).

Ultimately, 68.4% of the study subjects preferred aspart over lispro for CSII, with 15.8% preferring lispro and 15.8% with no preference. Aspart provided greater pump compatibility in terms of lower rates of catheter complications and dermal or subcutaneous irritations (66).

**CONCLUSION**

Although the clinical benefits of the RAIAs appear to be similar, studies have demonstrated aspart to have greater compatibility for use in insulin pumps in comparison with lispro or glulisine. In numerous studies, aspart demonstrated a rapid rate of absorption (14,19,47-49), stable postprandial control (15,39,52,54,55), resistance to isoelectric precipitation (43,59), and low rates of fibrillation (61,62) and occlusion (15,63-65) in CSII. These qualities make aspart compatible for use in CSII therapy and perhaps a better choice of insulin to ensure improved outcomes, which in turn may generate improved treatment adherence. Future long-term research on the use of RAIAs in CSII is warranted, including studies on pump compatibility and use in different patient populations, such as elderly patients and patients with type 2 diabetes.

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