Case Report

INSULIN GLARGINE AND INSULIN ASPART OVERDOSE WITH PHARMACOKINETIC ANALYSIS

Christian C.K. Kim, MD[1]; Thomas G. Rosano, PhD[2]; Erin E. Chambers, PhD[3]; Manjunath P. Pai, PharmD[4]; James Desemone, MD[1]

ABSTRACT

Objective: Insulin glargine is a long-acting insulin analogue with biotransformation, at the injection site, to a primary active metabolite (glargine-M1). Combined therapy with rapid-acting insulin aspart is common, but pharmacokinetics with supra-therapeutic doses have not been reported. We present clinical and pharmacokinetic findings for 2 episodes of overdose with glargine and aspart in a 43-year-old male with type 1 diabetes.

Methods: Liquid chromatography–tandem mass spectrometry was used to detect and quantitate the M1 and insulin aspart levels in the serum of an individual who administered large supra-therapeutic doses of these medications.

Results: Prolonged insulin analogue action was associated with measurable aspart levels in the circulation for the first day and with glargine-M1 for up to 5 days. Dextrose infusion rate correlated closely with the circulating levels of aspart and glargine-M1. Throughout the clinical treatment, glargine concentrations were near or below the threshold of detection. Several peaks of glargine metabolite were observed before it was completely undetectable. The pharmacokinetic profile of the simultaneous insulin aspart overdose was also prolonged.

Conclusion: This is the first report of a simultaneous overdose in insulin glargine and insulin aspart where direct serum measurements were made of insulin glargine, its active metabolite glargine-M1, and insulin aspart. This case demonstrates a substantial contribution of glargine-M1 to the severity and prolongation of hypoglycemic risk in glargine overdose. **(AACE Clinical Case Rep. 2016;2:e122-e128)**

Abbreviations:
Glargine-M1 = active metabolite glargine-M1; **IV** = intravenous; **LC-MS/MS** = liquid chromatography–tandem mass spectrometry; **LOQ** = limit of quantification

INTRODUCTION

Insulin glargine is a long-acting, slow tissue release insulin analogue that is designed to provide a steady profile of insulin in order to mimic the basal endogenous insulin release profile. The activity of this agent is dependent on biotransformation of glargine to the active metabolite, glargine-M1, which has been well characterized following therapeutic administration of insulin glargine (1-3) (Fig. 1). Overdose by subcutaneous injection with glargine has been reported, but the contribution of glargine-M1 metabolism to the prolonged episode of hypoglycemia following a supra-therapeutic dose of glargine is unknown (4-10). Recent development of a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for quantification of the insulin analogues (11), along with clinical treatment of 2 episodes of combined glargine and aspart overdose in a patient with diabetes, resulted in the following phar-

Submitted for publication February 21, 2015
Accepted for publication May 28, 2015
From the [1]Department of Medicine, Albany Medical College, Albany, New York, [2]Department of Pathology and Laboratory Medicine, Albany Medical Center Hospital, Albany, New York, [3]Waters Corporation, Milford, Massachusetts, and [4]Department of Health Sciences, Albany College of Pharmacy and Health Sciences, Albany, New York.
Address correspondence to Dr. Christian C.K. Kim, Albany Medical Center, Endocrinology, 25 Hackett Boulevard, Albany, NY 12208.
E-mail: vizcoz1@gmail.com.
DOI:10.4158/EP15689.CR

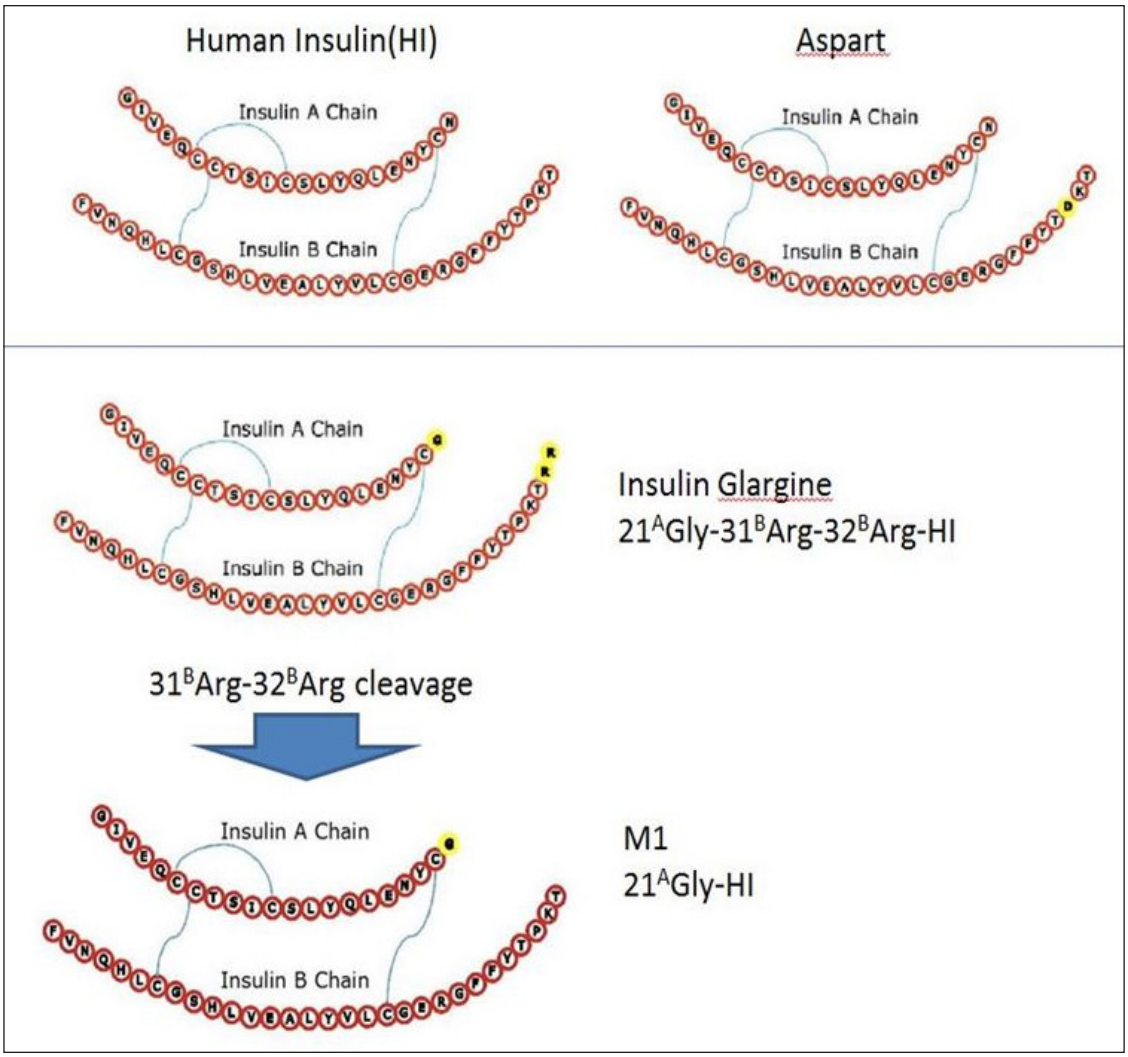

Fig. 1. Aspart is homologous with human insulin, with the exception of a single substitution of the amino acid proline by aspartic acid at the B28 position. Glargine is different from human insulin in that the amino acid asparagine at position 21 on the A-chain is replaced by glycine, and 2 arginine residues are added to the C-terminus of the B-chain. After subcutaneous injection, M1 is formed by enzymatic removal of the arginine pair at the B-chain.

macokinetic investigation of insulin analogue and metabolite levels following supra-therapeutic administration of glargine and aspart.

CASE REPORT

Case Description

A 43-year-old male weighing 119 kg, with a history of major depression and suicidal attempts, had a diagnosis of ketosis-prone type 1 diabetes mellitus for more than 20 years. He was brought to the emergency department from an outside mental-health facility after self-administering an overdose of insulin analogues. Upon arrival at the emergency department, the patient was lethargic, with a serum glucose level of 30 mg/dL. Electrolyte measurement revealed levels of sodium at 143 mEq/L, potassium at 3.0 mEq/L, chloride at 109 mEq/L, blood urea nitrogen at 23 mg/dL, creatinine at 1.1 mg/dL, and a glomerular filtration rate >60 mL/min/1.73m^2. Liver transaminases were within the reference range. The patient subsequently reported self-injecting 1,500 units of glargine and 600 units of aspart approximately 5 hours prior to admission. He administered the injections at 7 sites on his body, with 5 glargine and 2 aspart pens, delivering approximately 300 units of total insulin analogue at each site. The patient received multiple ampules of 50% dextrose (D50) in the first few hours of presentation and was placed on 20% dextrose in water (D20W) intravenous (IV) infusion at 200 mL/hour. His mental status continued to deteriorate until approximately 30 hours after admission, when his glucose administration requirements stabilized on continuous dextrose infusion and his overall condition improved. The IV infusion rate

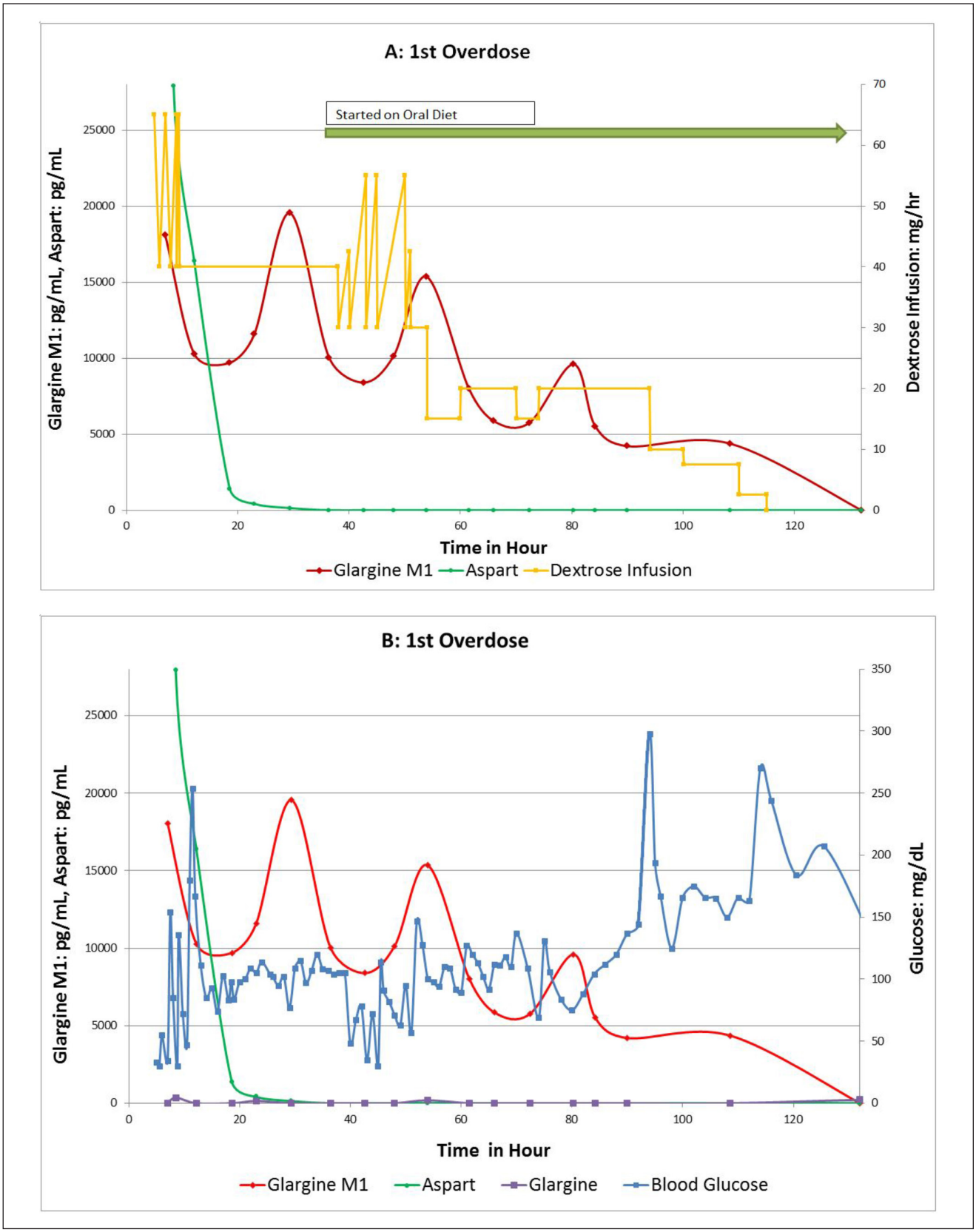

Fig. 2. First episode of overdose. *A*, Higher rates of dextrose infusion needed when the levels of glargine-M1 and aspart were high. *B*, Low blood glucose levels noted when the levels of aspart and glargine metabolite were high. Glargine levels were very low and did not significantly contribute to the hypoglycemia.

of dextrose was adjusted to maintain the blood glucose level in the normal range. About 35 hours after presentation, the patient was allowed to eat ad lib. Hypokalemia was corrected by IV and oral potassium replacement. Approximately 115 hours from the time of overdose, he no longer required IV dextrose infusion to prevent hypoglycemia. On the following day, his usual dose of 30 units of glargine at bedtime was resumed and several days later, 7 to 10 units of aspart was re-instituted with meals. The patient was transferred to an inpatient psychiatric unit and then discharged home 11 weeks later.

One day after being discharged from the inpatient psychiatric unit, he was found obtunded by the landlord at his apartment with 3 empty glargine and 2 empty aspart pens. Approximately 3 hours after the overdose injections, he was re-admitted to the same medical center for treatment of hypoglycemia. He admitted to injecting 900 units of glargine and 600 units of aspart. His electrolytes and liver function tests were unremarkable. D20W was started at 200 mL/hour. The lowest measured blood glucose level was 49 mg/dL at 8 hours after admission. His mental status improved immediately with dextrose infusion, and he ate liberally. The dextrose infusion rate was adjusted to prevent hypoglycemia, and the infusion was discontinued approximately 96 hours after the time of the overdose.

Research Design and Methods

Blood samples from clinical treatment were collected from the time of each hospital presentation at approximately 5-hour intervals until the risk of hypoglycemia abated. The protocol for collection and testing of discarded, de-identified specimens in the case was reviewed by the Institutional Review Board of the Albany Medical College. Serum samples were aliquoted and stored at −80°C within 12 hours of blood draw and were maintained at −80°C until analysis by LC-MS/MS for quantification of the glargine, glargine-M1, and insulin aspart levels. Blood glucose monitoring data were obtained from the clinical chart, and dextrose infusion rates were calculated based on the recorded values of continuous intravenous infusion. Rapid infusions of ampules of 50% dextrose are included in the dextrose infusion graph and are represented as "spikes" on Figures 2 *A* and 3 *A*. LC-MS/MS analysis for insulin analogues and glargine metabolite was performed according to a previously published method, using triple-quadrupole MS and specific multiple reaction monitoring (MRM) transitions as detailed therein (11). Briefly, the MRM transition for the M1 metabolite of glargine, which was not previously reported, was *m*/*z* 959.7 >136. In addition to glargine, glargine-M1, and insulin aspart, the presence of other insulin analogues (lispro, detemir, glulisine) and human insulin were also selectively tested. Clean-up of serum samples (250 μL) was performed by acetonitrile precipitation of high-abundance proteins, followed by mixed-mode strong anion exchange solid-phase extraction of the glargine-M1 and intact human insulin and analogues. At-column dilution and analyte trapping with back elution were employed to enhance analyte sensitivity and selectivity prior to high-resolution liquid chromatography separation. Glargine-M1 reference material was generated through sequential enzymatic cleavage of the two terminal arginines from the glargine B-chain using carboxypepsidase B and incubation at 37°C for 45 minutes. For insulin analogues, levels of detection ranged between 50 and 200 pg/mL; accuracy ranged from 94 to 98%; average inter- and intraday precision was 7.5 and 5.3%, respectively; and matrix effect was <15%. For the insulin glargine-M1 and aspart, the limit of quantification (LOQ) was 200 pg/mL. For insulin glargine, the LOQ was 200 pg/mL.

RESULTS

The profile of dextrose infusion rate, glargine-M1, and aspart are shown in Figure 2 for the first overdose and Figure 3 for the second overdose. The first overdose required a total of 115 hours of dextrose infusion (Fig. 2 *A*). The aspart level was highest (76,460 pg/mL) in the first blood collection during the admission, which was about 7 hours after the time of the overdose (this is not shown in the figure because the value is out of the scale). The aspart concentration rapidly decreased over the next 19 hours and then plateaued at concentrations greater than 100 pg/mL, until becoming undetectable at 29 hours after the overdose. Glargine-M1 was also found at a high concentration (18,070 pg/mL) in the serum at the time of presentation. Glargine-M1 concentration then decreased over the next several hours but started to increase again, reaching a peak with maximal concentration of 19,545 pg/mL at 30 hours. Two more peaks with lower maximal concentration of 15,359 pg/mL (at 54 hours) and 9,601 pg/mL (at 80 hours) were determined. The glargine-M1 level then gradually decreased but was measurable up to 109 hours after the overdose (extrapolated data points extend up to 132 hours, when the M1 concentration was undetectable). High levels of aspart and glargine-M1 correlated with both higher rates of dextrose infusion and low blood glucose levels (Fig. 2 *B*).

The results of the second overdose are shown in Figure 3. Treatment for hypoglycemia during this episode required dextrose infusion for 96 hours (Fig. 3 *B*). The aspart concentration was highest in the first several hours of presentation and measurable up to 22 hours after the overdose. Glargine-M1 had a maximum concentration of 9,960 pg/mL at 11 hours post-overdose, with progressively lower peak concentrations observed at 28 and 68 hours. The level was detected up to 70 hours (extrapolated data points extend up to 74 hours, when the M1 concentration was undetectable). As in the first episode, high concentrations of aspart and glargine-M1 correlated inversely with the low blood glucose level (Fig. 3 *B*) and directly with dextrose infusion rate (Fig. 3 *A*).

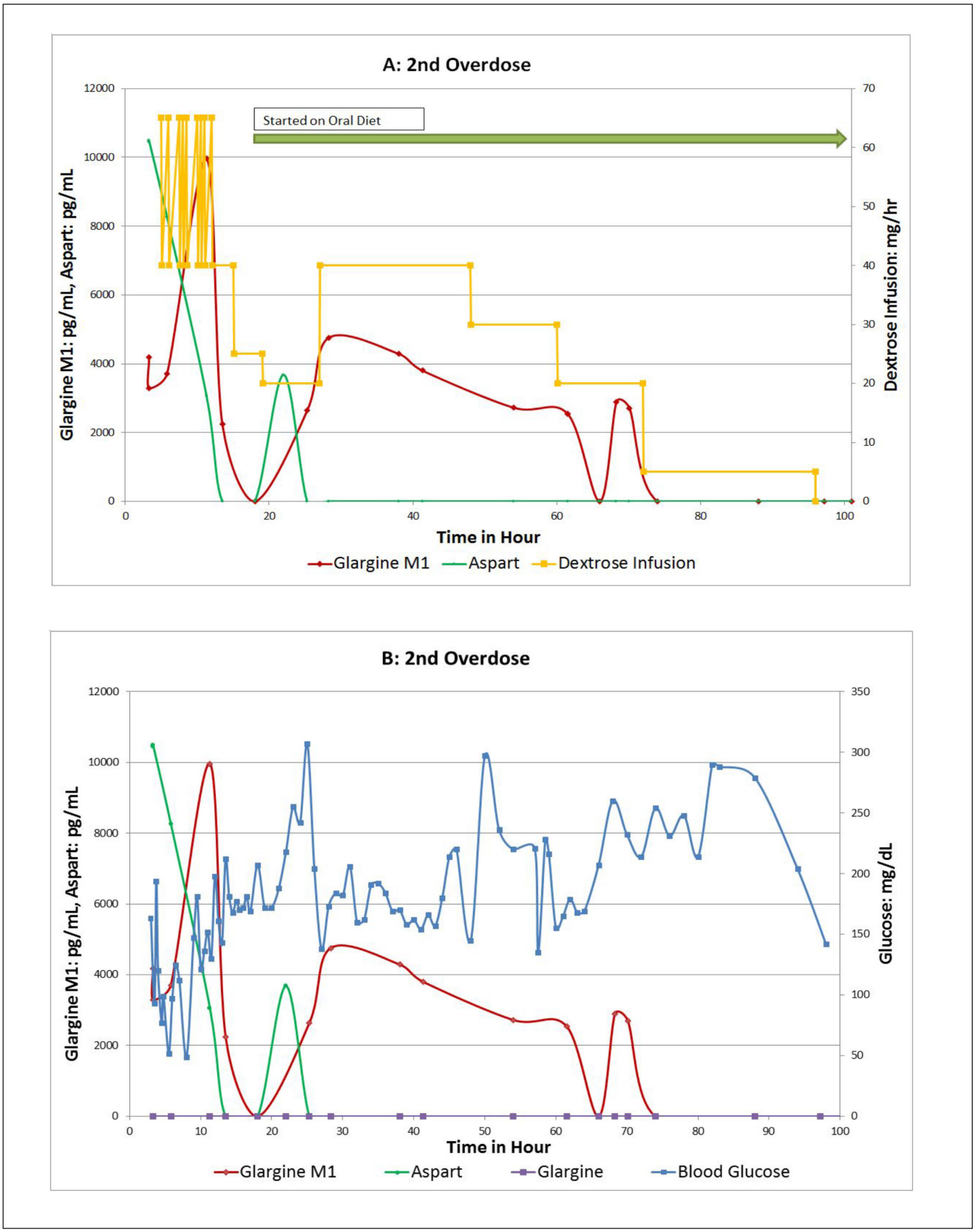

Fig. 3. Second episode of overdose. *A*, Higher rates of dextrose infusion needed when the levels of glargine-M1 and aspart were high. *B*, Low blood glucose levels noted when the levels of aspart and glargine metabolite were high. Glargine was undetectable.

On the first overdose, glargine level was detected at 8, 23, 54, and 108 hours. The concentrations were all below 400 pg/mL (Fig. 2 *B*). On the second overdose, glargine level was not detected (Fig. 3 *B*).

DISCUSSION

We present a unique patient who took overdoses of simultaneously administered insulin glargine and insulin aspart on two separate occasions. The results of this study indicate that the circulating levels of aspart and glargine-M1 are the major hypoglycemic agents circulating in blood in cases during a combined aspart and glargine overdose. High aspart levels in the first few hours contributed significantly to the hypoglycemia illustrated in Figures 2 *B* and 3 *B*, and high rates of dextrose infusion and multiple ampules of D50 were required to prevent hypoglycemia. After the aspart concentration was undetectable, the glargine-M1 concentration alone was significant enough to cause hypoglycemia. Both the aspart and glargine-M1 had additive effects on the glucose lowering, and thus, the dextrose infusion requirement.

The therapeutic reference range for M1 has not yet been identified, but according to the report by Bolli et al (2), when glargine was administered at a therapeutic dose, 0.3 units/kg, the maximum concentration of M1 was 470 pg/mL. In our patient, the maximum concentration of M1 was well over 19,000 pg/mL on the first overdose and almost 10,000 pg/mL on the second overdose.

In a number of previously reported cases involving insulin analogues, overdoses were associated with an extended duration of action compared to pharmacokinetics seen with therapeutic doses (4-10). The two episodes of overdose in our patient also showed a risk for hypoglycemia that was longer than the duration of action of either insulin glargine or insulin aspart when given at therapeutic doses. We also found that prolonged hypoglycemic effects correlated with prolonged glargine-M1 and insulin aspart concentrations in serum. This highlights the importance for clinicians to monitor hypoglycemia in patients who receive excessive or supra-therapeutic, subcutaneous doses of these insulin analogues.

Our findings also support the work by Groth et al (10), who showed that the amount of analogue dose correlated positively with the duration of risk of hypoglycemia. Additionally, our data suggest that circulating glargine-M1 plays a major role in the prolonged hypoglycemic risk following a glargine overdose. This study also shows that the circulating glargine concentration in blood remained near or below the analysis LOQ during the prolonged period of hypoglycemic risk, suggesting that circulating parent drug is not a significant contributor to the clinical risk of hypoglycemia. Although lower doses of insulin glargine were used (up to 1.2 units/kg), Bolli et al (2) describe similar findings.

Despite the results of the ORIGIN trial (12), where insulin glargine had a neutral association with the overall and cancer-specific outcomes, some clinicians may be concerned about the malignant potential of very large supra-therapeutic doses of glargine. This concern stems from the increased binding affinity of glargine to the insulin-like growth factor 1 (IGF-1) receptor. If such a concern exists, the negligible level of circulating glargine reported in this study should be re-assuring. It should also be re-assuring that glargine-M1 exhibits lower binding affinity to the IGF-1 receptor than human insulin (2).

Supra-therapeutic doses of insulin glargine administered via a subcutaneous tissue depot appear to not only alter the duration of the hypoglycemic action but also results in an oscillating pattern of circulating glargine-M1 concentration that influences dextrose infusion requirements during therapy. The observed multiphasic peak and trough profiles of glargine-M1 concentration in serum have not been previously described. This pharmacokinetic profile may have been influenced by multi-site injections of insulin glargine, the rate of conversion of glargine to glargine-M1, and injection site–specific rates of entry of glargine-M1 into the systemic circulation. The repeated observation of the glargine-M1 profile on two occasions with a more blunted peak in the second episode supports this conclusion.

CONCLUSION

This study highlights the potential clinical application of glargine-M1 assay as an aid in prediction of the duration of sustained hypoglycemic risk in cases of overdose. Further investigation of the profile and correlation of glargine-M1 concentrations with hypoglycemic risk in cases of overdose are necessary to validate these findings. In addition, the results of this case study suggest the value of glargine-M1 analysis and the need for further investigation of glargine-M1 levels with therapeutic use of glargine.

DISCLOSURE

The authors have no multiplicity of interest to disclose.

REFERENCES

1. **Kuerzel GU, Shukla U, Scholtz HE, et al.** Biotransformation of insulin glargine after subcutaneous injection in healthy subjects. *Curr Med Res Opin*. 2003;19:34-40.
2. **Bolli GB, Hahn AD, Schmidt R, et al.** Plasma exposure to insulin glargine and its metabolites M1 and M2 after subcutaneous injection of therapeutic and supratherapeutic doses of glargine in subjects with type 1 diabetes. *Diabetes Care*. 2012;35:2626-2630.
3. **Varewijck AJ, Yki-Järvinen H, Schmidt R, Tennagels N, Janssen JA.** Concentrations of insulin glargine and its metabolites during long-term insulin therapy in type

2 diabetic patients and comparison of effects of insulin glargine, its metabolites, IGF-1, and human insulin on insulin and IGF-1 receptor signaling. *Diabetes*. 2013;62:2539-2544.

4. **Fromont I, Benhaim D, Ottomani A, Valéro R, Molines L, Vialettes B.** Prolonged glucose requirements after intentional glargine and aspart overdose. *Diabetes Metab*. 2007;33:390-392.
5. **Fuller ET, Miller MA, Kaylor DW, Janke C.** Lantus overdose: case presentation and management options. *J Emerg Med*. 2009;36;26-29.
6. **Lu M, Inboriboon PC.** Lantus insulin overdose: a case report. *J Emerg Med*. 2011;41:374-377.
7. **Doğan FS, Onur ÖE, Altınok AD, Güneysel Ö.** Insulin glargine overdose. *J Pharmacol Pharmacother.* 2012;3:333-335.
8. **Mork TA, Killeen CT, Patel NK, Dohnal JM, Karydes HC, Leikin JB.** Massive insulin overdose managed by monitoring daily insulin levels. *Am J Ther.* 2011;18:e162-e166.
9. **Warriner D, Debono M, Gandhi RA, Chong E, Creagh F.** Acute hepatic injury following treatment of a long-acting insulin analogue overdose necessitating urgent insulin depot excision. *Diabet Med*. 2012;29:232-235.
10. **Groth CM, Banzon ER.** Octreotide for the treatment of hypoglycemia after insulin glargine overdose. *J Emerg Med*. 2013;45;194-198.
11. **Chambers EE, Fountain KJ, Smith N, et al.** Multidimensional LC-MS/MS enables simultaneous quantification of intact human insulin and five recombinant analogs in human plasma. *Anal Chem*. 2014;86:694-702.
12. **Bordeleau L, Yakubovich N, Dagenais GR, et al.** The association of basal insulin glargine and/or n-3 fatty acids with incident cancers in patients with dysglycemia. *Diabetes Care*. 2014;37:1360-1366.