Instability of Insulin Aspart Diluted in Dextrose

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Insulin aspart (Novorapid) is a rapid-acting insulin analog that is maintained in hexameric form by phenolic preservatives (phenol and metacresol) and stabilized by insulin-zinc complex. According to the manufacturers, insulin can be independently diluted either in 5% dextrose solution (D5%) or 0.9% sodium chloride solution. In reality, in most clinical units, such as the neonatal intensive care unit (NICU), insulin aspart is more frequently diluted in D5% than in saline solution due to sodium restriction.

However, injectable dextrose solutions contain glucose degradation products (1) that are capable of binding proteins—like insulin—through a glycation phenomenon. Therefore, dilution of Novorapid in D5% would be more likely to cause glycated insulin and may raise a worrisome problem in terms of insulin stability. The objective of this study was to investigate the stability of insulin aspart diluted in D5% at both adult and neonate therapeutic concentrations in order to ensure its appropriateness in the preparation of insulin aspart.

To address this issue, insulin aspart diluted in D5% was assayed by a stability-indicating method using high-performance liquid chromatography (HPLC) coupled with an ultraviolet (UV) detector. The nature of the formed product was determined by HPLC coupled with a mass spectrometer. The effect of insulin and dextrose contact time on insulin stability was also studied by HPLC-UV, and four replicates were checked every hour for 24 h and at 48 h, 72 h, and 168 h in a thermostatic chamber at 25°C.

In HPLC-UV, phenol, metacresol, and insulin aspart were eluted at 2.63, 4.14, and 5.55 min, respectively. After diluting insulin aspart in D5% at concentrations ranging from 0.1 to 1 unit/mL, we noted a fourth signal eluted at 7.32 min. HPLC–mass spectrometry analysis revealed that this fourth signal corresponded to monoglycated insulin. HPLC-UV analysis showed that the increase of glycated insulin was related to an increase in contact time between insulin and dextrose in the first 24 h (Fig. 1). In parallel, there was a decrease of the native form of insulin aspart. At $t = 24$ h, the glycated insulin level reached 30.2 $\pm$ 0.5% of the initial concentration of insulin aspart at 1 unit/mL. From 24 h to 1 week, there was a stabilization of both amounts and glycated insulin remained relatively stable. Regarding this stabilization, we can make two hypotheses. First, a reaction could occur involving a glucose degradation product whose concentration is low compared with insulin aspart, such as 3-deoxyglucosone. A second hypothesis could be that the formed product corresponds to the intermediate Schiff base through a reversible reaction, leading to the final Amadori product by an irreversible slow rearrangement reaction.

Considering previously published data, our investigations are, to our knowledge, the first to highlight the phenomenon of glycation for insulin aspart. The question addressed in this study is therefore two-pronged: is the use of insulin aspart diluted in dextrose adapted to treat both neonates and adults? The major weakness of this work is that the biological activity of glycated insulin aspart has not yet been evaluated. Currently, we do not know if and how this biological activity is affected. Several studies have reported glycation of other types of insulin and argued that glycated insulin may present altered biological activity with a decrease in its hypoglycemic activity (2,3) and that it may play a significant contributory role in insulin resistance and glucose intolerance in type 2 diabetes (4,5). Administration of partially glycated insulin may therefore be one of the various sources of glycemic variability in patients because of potential altered biological activity and

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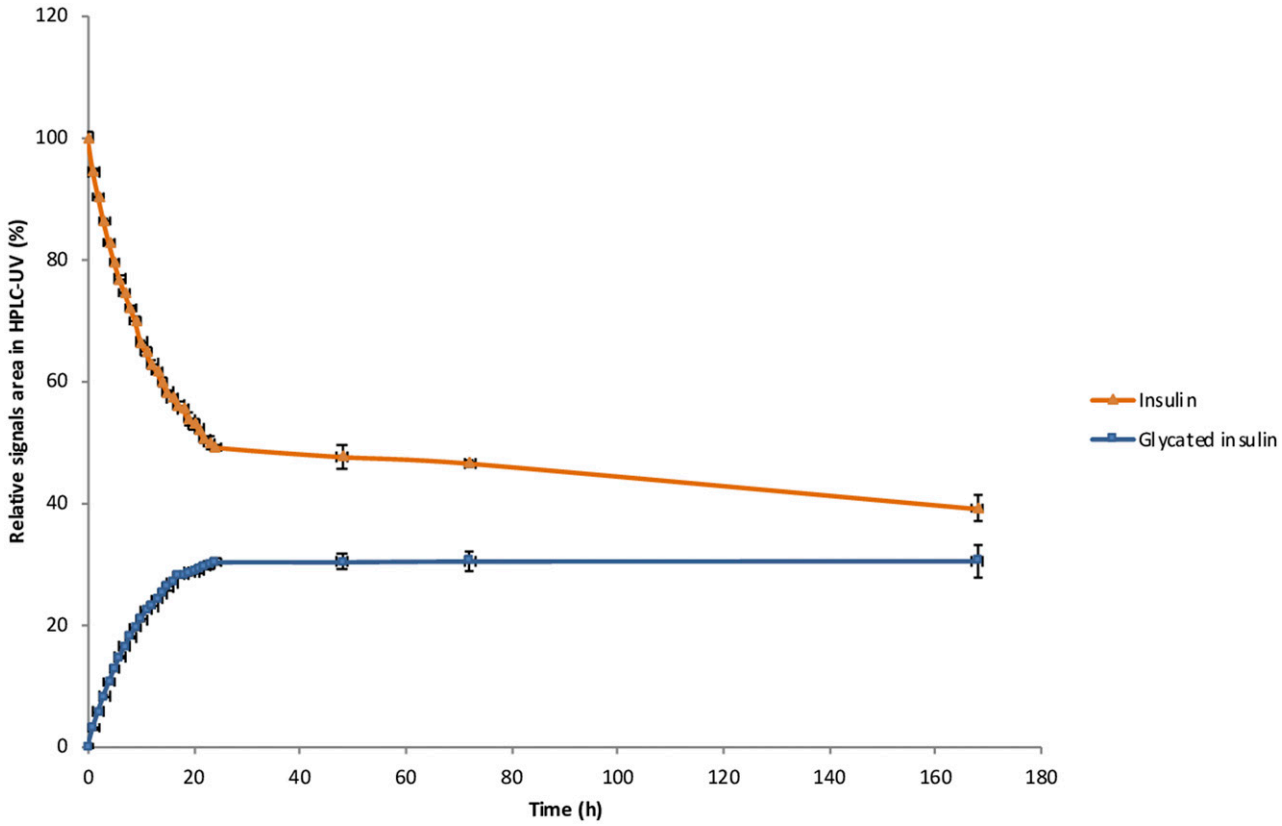

Figure 1—Evolution of signals areas for insulin and glycated insulin ($n = 4$) relative to the initial amount of insulin aspart, for insulin aspart diluted in D5% at 1 unit/mL at 25°C. The blue line corresponds to the evolution of average areas ± SDs for glycated insulin, and the orange line corresponds to the evolution of average areas ± SDs for insulin aspart.

because of a loss of native insulin aspart compared with the expected concentration. This phenomenon will be more impactful for neonates than for adults.

No data are currently available about the stability of insulin aspart diluted with dextrose. The only standard available is the summary of product characteristics for insulin aspart in which the laboratory allows for free choice of diluent between saline, D5%, or D10%. In view of our results, the choice of D5% or any other concentration with dextrose as a diluent seems debatable.

For preparations of insulin in the ward, it would be possible to adopt dilution in saline, which therefore eliminates the phenomenon of glycation. In the NICU, sodium intake and output can be calculated for each newborn and balanced by decreasing intakes in parenteral nutrition to limit the risk of sodium overload. Thus, with the current state of knowledge, it seems appropriate to recommend saline diluent to optimize the efficacy of insulin aspart treatment and to limit glycemic variability.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.
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