Glargine and Cancer: Can We Now Suggest Closure?

The two publications on the pharmacokinetics of insulin glargine in individuals with type 1 and type 2 diabetes in this issue of *Diabetes Care* (1,2) provide additional explanatory evidence in support of the definitive findings in the Outcome Reduction with Initial Glargine Intervention (ORIGIN) study in which exposure to insulin glargine for a median duration of 6.2 years did not increase the risk of any cancer (hazard ratio 1.00 [95% CI 0.88–1.13]) or death from cancer (0.94 [0.77–1.15]) (3).

Soon after glargine's long awaited and welcome introduction into clinical practice in 2000, questions were raised about its safety profile. This conception was based on the early finding that insulin glargine had an enhanced affinity to IGF-1 receptors when tested in a human osteosarcoma cell line (Saos/B10) with a preponderance of IGF-1 receptors and associated with increased mitogenicity (proliferation in an existing tumor cell line) (4). These findings were akin to those observed with the AspB10 insulin analog, the development of which had earlier been discontinued because of an increase in both benign and malignant mammary gland tumors in Sprague-Dawley rats after 12 months' exposure (5,6); thus AspB10 was referred to as the "carcinogenic insulin analog" (7). In contrast, detailed extensive toxicological lifetime carcinogenicity studies of insulin glargine in animals (rodent and nonrodent), albeit at lower doses, revealed no carcinogenicity signal with insulin glargine when compared with human insulin (8,9). The risk was regarded as small by the European Medicines Agency safety working party because of the lack of effect on mammary gland proliferation, absence of mammary carcinoma, and rare tumors during the lifetime studies in animals. The higher insulin receptor affinity and consequent prolonged dephosphorylation due to the increased residence time of AspB10 on insulin receptors (4) along with a predilection for the insulin receptor IR-A isoform (10) may in large part account for the differential metabolic and mitogenic outcomes observed when compared with human insulin and insulin glargine.

Di-arginyl insulin (ArgB31-ArgB32 human insulin) is known to be an intermediate in the conversion of proinsulin to insulin. Insulin glargine (GlyA21-ArgB31-ArgB32 human insulin) possesses glycine at A21 instead of asparagines, thereby adjusting its solubility at neutral pH for retarded release while lowering its propensity for aggregation. After subcutaneous administration, insulin glargine precipitates amorphously and is then slowly released according to zero-order kinetics. It has been documented for some time that insulin glargine is biotransformed both in the subcutaneous tissue and circulation (11). Sequential cleavage of the carboxyl terminus of the C-chain occurs via local and systemic converting proteases into the primary metabolite GlyA21 human insulin (M1) and also GlyA21 des-ThrB30 human insulin (M2) as described using high-performance liquid chromatography and a nonspecific radioimmunoassay. Biotransformation in serum occurs rapidly with ~70% of insulin glargine converted to M1 within 30 min of incubation (12,13). The metabolites M1 and M2 retain the full biological activity of human insulin and have substantially reduced IGF-1 receptor binding and mitogenic potency relative to human insulin (4). Whereas insulin glargine has a greater affinity to IGF-1 receptors than human insulin (10-fold) it has a 100-fold lower affinity than native IGF-1 (14). This enhanced IGF-1 affinity is reversed by removal of the di-arginyl molecules. The primary (M1) and secondary (M2) metabolites are less metabolically active with reduced affinity to IGF-1 receptors and equivalent growth-promoting activity in Saos-2 and MCF-7 cell lines compared with human insulin (15).

The early vital information on the biotransformation of insulin glargine (11) was seemingly ignored for many years, with research groups having chosen instead to focus on the in vitro IGF-1 binding properties of the parent compound insulin glargine. Therefore, little further development has occurred until the current articles by Bolli and colleagues (1,2). In the meantime, researchers have extensively explored the potential adverse clinical impact of insulin glargine on diabetic retinopathy and cancer.

Concerns relating to the development or progression of diabetic retinopathy by insulin glargine were alleviated when in both a meta-analysis of four phase 3 trials (16) and a randomized control trial over a 5-year period revealed no difference between NPH and insulin glargine (17). In September of 2009, four international communications relating to insulin and the risk of cancer were published together (18–21); three were instigated by the European Association for the Study of Diabetes to explore the validity of the first study carried out in Germany (18), which demonstrated a strong correlation between insulin dose and cancer risk and implied that insulin glargine carried a higher risk than human insulin. This lead to considerable anxiety among the insulin-treated diabetic population and the diabetes care community alike, which necessitated the regulatory authorities and diabetic associations to issue statements of reassurance. The findings of Hemkens et al. (18) were subsequently criticized because they used an unconventional and fundamentally flawed analysis that adjusted for insulin dosage, which meant that the conclusions were unsupportable (22). There was no excess cancer risk seen in a 5-year randomized control trial comparing NPH and insulin glargine (17) or when the combined randomized control trial experience of malignancies in studies using insulin glargine were evaluated (23). With regard to the general clinical question, the Consensus Report on Cancer and Diabetes by the American Diabetes Association and American Cancer Society concluded that further research was needed to clarify the relationship between exogenous insulin and increased cancer risk and the specific question relating to insulin glargine (24). Sandow (9) attempted to highlight the complexity of the relationship between the growth effects of insulin and insulin analogs while emphasizing the need for clarity regarding the meaning of mitogenicity under physiological and pathophysiological situations in relationship to insulin and insulin analogs.

In hindsight, it is somewhat unfortunate that during the discussion of insulin glargine, after its introduction into clinical

practice, the published results of the toxicological studies that demonstrated no biological (mitogenic) signal in the lifetime studies in animals (at maximum tolerated doses), which could be attributed to the slightly enhanced IGF-1 receptor affinity, received little attention. The concept of the di-arginyl insulins mimicking nature with the retarding principle residing in the molecule itself (solubility) in contrast to molecules requiring protamine, excess zinc or, more recently, acylated fatty acids was also seemingly forgotten. It is only relatively recently that this latter aspect has been revised after the in vitro studies, which confirm the reduced IGF-1 receptor binding and low mitogenic potency of the active metabolites (M1 and M2) of insulin glargine. Therefore, the delay in fully exploring the pharmacokinetics of insulin glargine in humans after the early studies with limited methodologies by Kuerzel et al. (11) and Agin et al. (12) has been most unfortunate. Because of technical constraints, a detailed description of the pharmacokinetics of insulin glargine and its metabolites (M1 and M2) in humans has hitherto not been possible. This was due to cross-reactivity between insulin glargine and its primary metabolite in the radioimmunoassays then used (25).

The long-awaited need for more comprehensive pharmacokinetic investigations of insulin glargine in humans is addressed by the two accompanying articles by Bolli and colleagues (1,2). From a clinical point of view, it is essential to provide information about the circulating concentrations of the parent compound and its active metabolites using adequate methodologies. These two studies present the first such data in individuals with both type 1 (1) and type 2 (2) diabetes using a newly developed specific assay method involving liquid chromatography-tandem mass spectrometry capable of providing discrete measurements of insulin glargine and its metabolites M1 and M2 after their extraction from human plasma using immunoaffinity columns. Insulin glargine was administered to individuals with type 1 diabetes by bolus subcutaneous injection at both therapeutic and supratherapeutic doses (0.3, 0.6, and 1.2 units/kg) in a euglycemic clamp used to define the glucodynamic changes over the study period of 30 h. Each participant received a single dose of insulin glargine. The parent compound, insulin glargine, and the metabolite M2 were rarely detected in plasma above the lower limit of detection at 33 pmol/L (~6 μU/mL) regardless of the dose (up to 1.2 units/kg). M1 was detected in plasma in a dose-related fashion correlating with the observed glucodynamic changes observed. Importantly, insulin glargine was not detected in plasma at the higher dosage. Essentially similar findings were seen in the type 2 study after the administration of insulin glargine at only a single dose of 0.4 units/kg, with the metabolite M1 being predominant in the plasma with little or no parent insulin glargine or the second metabolite M2 detectable by involving liquid chromatography-tandem mass spectrometry (2). A similar plasma insulin profile was observed when the radioimmunoassay was used, indirectly confirming the major contribution by the M1 metabolite. There is acknowledgment by the authors that it is also necessary and advisable to examine the metabolism of insulin glargine after prolonged exposure and at even higher doses, which are sometimes used in obese and insulin-resistant individuals.

Therefore, the virtual absence of the parent compound insulin glargine in the circulation after its subcutaneous injection invalidates the submission that the in vitro findings of enhanced IGF-1 binding and mitogenicity of insulin glargine has a clinical correlate, especially as insulin glargine is extensively and quickly metabolized in the subcutaneous tissue and in the systemic circulation to its metabolites M1 and M2, both of which have lesser metabolic and similar mitogenic potency to human insulin (15,26).

The important and definitive findings by Bolli and colleagues (1,2) represent a critical piece of evidence in support of the recent findings of the ORIGIN trial (3) and the French National Healthcare Insurance Database (27), neither of which observed an excess risk of cancer during long-term exposure to insulin glargine. It is, however, surprising that it has taken almost 12 years since insulin glargine was first introduced to start to truly understand its metabolic fate in humans after subcutaneous administration despite the early observations by Kuerzel et al. (11).

In response to concerns raised by the laboratory findings of Kurtzhals et al. (4) and the flawed interpretation of the epidemiological study triggered by a German health insurance initiative based on reimbursement considerations (18), a large volume of clinical and experimental data have been generated during this intervening, diversionary period. Research addressing the question whether diabetes and/or diabetes therapy influences the risk of cancer has since provided extensive and invaluable information on the complex interrelationship between diabetes, its treatment, and the various types of cancers. However, on the basis of the findings from epidemiological studies (3,27) and the latest welcome and overdue pharmacokinetic data (1,2)—which is further supported by similar findings in young children (28)—the chapter on whether insulin glargine per se is an independent risk factor for cancer should now be closed.

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