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Stable Liquid Glucagon: Beyond Emergency Hypoglycemia Rescue

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Abstract

Glycemic control is the mainstay of preventing diabetes complications at the expense of increased risk of hypoglycemia. Severe hypoglycemia negatively impacts the quality of life of patients with type 1 diabetes and can lead to morbidity and mortality. Currently available glucagon emergency kits are effective at treating hypoglycemia when correctly used, however use is complicated especially by untrained persons. Better formulations and devices for glucagon treatment of hypoglycemia are needed, specifically stable liquid glucagon. Out of the scope of this review, other potential uses of stable liquid glucagon include congenital hyperinsulinism, post–bariatric surgery hypoglycemia, and insulinoma induced hypoglycemia. In the 35 years since Food and Drug Administration (FDA) approval of the first liquid stable human recombinant insulin, we continue to wait for the glucagon counterpart. For mild hypoglycemia, a commercially available liquid stable glucagon would enable more widespread implementation of mini-dose glucagon use as well as glucagon in dual hormone closed-loop systems. This review focuses on the current and upcoming pharmaceutical uses of glucagon in the treatment of type 1 diabetes with an outlook on stable liquid glucagon preparations that will hopefully be available for use in patients in the near future.

Keywords

dual hormone artificial pancreas, glucagon, hypoglycemia, intranasal glucagon, mini-dose glucagon, type 1 diabetes

Glucagon and Hypoglycemia Physiology

The relationship of glucagon and insulin is a quintessential example of homeostasis.[1-3] Insulin binding to its receptor leads to a hypoglycemic effect via insertion of GLUT4 glucose transporters allowing circulating glucose to enter into cells. Glucagon works with epinephrine, cortisol and growth hormone to halt hypoglycemia. The main target tissue of glucagon is the liver, where its seven transmembrane G-protein coupled receptor is found. Intracellular signaling occurs through cAMP, resulting in glycogenolysis and gluconeogenesis to raise circulating glucose levels within 10-30 minutes.[4,5]

In diabetes, repeated bouts of hypoglycemia lead to reduced capacity to respond to future hypoglycemia, termed hypoglycemia-associated autonomic failure.[6-9] Longer duration of diabetes leads to blunted or absent glucagon response to hypoglycemia resulting in impaired and prolonged glucose recovery after hypoglycemia.[9] Hypoglycemia-associated autonomic failure also lowers the glucose threshold before there is an epinephrine surge.[10] Without this counterregulatory hormone response, patients lack symptoms of hypoglycemia, termed hypoglycemia unawareness. Hypoglycemia unawareness is associated with a high frequency of severe hypoglycemia, especially during sleep.[11] Exercise leads to decreased epinephrine response to hypoglycemia, also contributing to recurrent hypoglycemia.[8]

Negative Effects of Hypoglycemia

The ability to prevent long-term complications with aggressive glycemic control is limited by hypoglycemia.[12,13] Patients with type 1 diabetes average two episodes of symptomatic hypoglycemia per week.[10] Treatment of mild hypoglycemia requires consumption of extra calories; 16 g of glucose tabs contain 60 kcal, which is a significant downside for the greater than 50% of adults with type 1 diabetes that are overweight or obese.[14] Severe hypoglycemia is an episode requiring external assistance for recovery. In the T1D Exchange database, 19% of participants reported having severe hypoglycemia in past year.[15] Severe hypoglycemia is associated with car accidents, seizures, and coma.[15-17] Two to four percent of deaths in type 1 diabetes are attributed directly to hypoglycemia.[6,10,18] Rates of severe hypoglycemia in type 2 diabetes are estimated at 1 for every 10 of those in type 1 diabetes, however with nearly 10% of the US population (30.4 million people) affected by type 2 diabetes; the

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numbers become significant.[19] Fear of hypoglycemia may result in strained interpersonal relationships, reduced quality of life and loss of productivity.[20-24] Hypoglycemia remains a significant problem deserving effective treatment approaches. Currently, treatment is limited to ingestion of carbohydrates for mild hypoglycemia or intramuscular injection of reconstituted lyophilized glucagon for severe hypoglycemia.

Hypoglycemia Rescue: Current Formulations

When an unconscious person with diabetes cannot take in carbohydrates, currently parenteral glucagon is the only FDA-approved method of treatment for hypoglycemia outside of emergency medical care. There are currently two formulations of glucagon approved by the FDA for clinical use in the United States: GlucaGen HypoKit (Novo Nordisk, Copenhagen, Denmark) and Glucagon Emergency Kit (Eli Lilly, Indianapolis, IN).[4,5] Both of these commercially available products come as two-part kits containing a syringe prefilled with a proprietary solution of sterile water and vial with lyophilized powder. To prepare the glucagon for injection, a sharps cover is removed from the large gauge needle, the cap is removed from the vial, and then the solution is injected into the vial of powder and swirled gently until the solution is "clear and of water-like consistency."[4,5] When mixed, there is 1mg of glucagon in a 1 mL solution. The fluid is then drawn back up into the syringe and delivered intramuscularly into upper arm, thigh or gluteus. For adults and children weighing more than 44-55 lbs, the advised dose is 1mg, the full contents of the kit. For children less than age six or less than this weight, the advised dose is 0.5 mg. The solution should be used immediately and any remaining portion must be discarded immediately. Before reconstitution the kit should be stored at room temperature (20-25°C). The expiration date is 24 months after the date of manufacture.[25] The cash price for a glucagon kit as of October 2017 is $295-360 per single use emergency kit (goodrx.com,uptodate.comlexicomp).

Hypoglycemia Rescue: Intranasal Glucagon

The need for alternative routes for glucagon delivery beyond emergency kits is emphasized by a recent simulation study of injectable glucagon where 50% of caregivers and 80% of acquaintances failed to inject any glucagon and three participants delivered insulin instead of glucagon.[26] Intranasal medication administration, with its needle free design, is effective for use by bystanders in other emergency situations.[27] Intranasal glucagon results in lower blood glucagon concentrations than intramuscular glucagon, however clinically relevant glucose raising effects are similar to injection despite the lower doses delivered.[28] There is one intranasal product in stage 3 clinical trials; AMG504-1 (developed by Locemia Solutions, Montreal, Canada, now acquired by Eli Lilly).[29] In the research setting, intranasal glucagon had markedly better rates and time to delivery of the full glucagon dose as compared to usual emergency glucagon kits. When the intranasal glucagon was used by untrained bystanders, 94% delivered a full dose of intranasal glucagon with mean time less than 1 minute, while only 20% delivered a partial dose of intramuscular glucagon taking a mean time of 2.4 minutes.[26] Real-world use of intranasal glucagon is likely to be more cumbersome, especially in situations where the person is combative or having a seizure.

Hypoglycemia Rescue: Alternative Methods

Another logical solution is an automatic injector that premixes the lyophilized glucagon and then allows for direct injection without the numerous steps of current emergency kits. Enject (Battle Ground, WA) was moving toward this goal back in 2015 with its GlucaPen device, however this company is no longer active. (Personal communication) Zosano Pharma ZP-Glucagon patch (Fremont, CA) is a transdermal microneedle patch system with promising phase 2 trials in 2015,[30] yet as of March 2016 the company suspended its glucagon program. (Personal communication) The field is now moving toward glucagon in a stable liquid form to be used in existing pen devices for hypoglycemia rescue.

Mini-Dose Glucagon

As alternate routes of glucagon administration advance the treatment of severe hypoglycemia, the subcutaneous route offers promise in the treatment of mild hypoglycemia. Mini-dose glucagon refers to the use of low dose glucagon via the subcutaneous route. This therapy was initially reported in 2001.[31] It is widely accepted for mild hypoglycemia treatment in "sick day" protocols for children.[32] Mild episodes of hypoglycemia when oral carbohydrate intake is prohibitive (such as with an unwilling child or with a nausea/vomiting illness) can be easily remedied with this method. A standard glucagon rescue kit is diluted per package instructions, then the liquid is drawn up in a standard insulin syringe and a small dose of glucagon is delivered subcutaneously. The recommend dose increases with the age of the child; >2 years of age get 2 "units" (20 μg), 1 unit/year up to 15 "units" (150 μg). If the blood glucose does not improve by 15-30 minutes, then the dosage is doubled. In an outpatient pediatric series with mini-dose glucagon, 85% of episodes of hypoglycemia with inability/refusal to take oral carbohydrates were successfully managed at home.[33] The familiarity with insulin syringes and regain of control by the caretakers are major advantages of this approach. Major limitations include the price of glucagon emergency kits and degradation of glucagon within 24 hours of reconstitution necessitating disposal of the unused portion.

Some patients continue to use reconstituted glucagon beyond 24 hours; the risks of this are not known. Fortunately, nausea and vomiting side effects of glucagon are rarely seen at low dosage.[31,34-36]

Mini-dose glucagon is now being studied in adults. A recent study showed comparable efficacy of 150 μg of subcutaneous glucagon to 16 g of glucose tabs to treat hypoglycemia in an ambulatory setting.[36] With blood sugars 50-69 mg/dl, the clinically judged efficacy was 94% for glucagon versus 95% for glucose tabs. Failures to resolve hypoglycemia were associated with higher insulin on board at the time of the episode. Mini-dose glucagon also shows promise in preventing exercise-induced hypoglycemia in adults.[37] The main adverse events reported with mini-dose glucagon are nausea and vomiting which are rare and dose dependent.[31,34-36] To date, studies of mini-dose glucagon have been short in duration; we will need future studies to assess the risks of long term mini-dose glucagon use. Current glucagon kits are expensive especially in comparison to the cost of glucose tablets. It is clear a novel glucagon formulation is required to facilitate more widespread use of mini-dose glucagon.

Glucagon in Closed-Loop Systems

Automated insulin delivery systems, termed closed-loop systems, are now possible with the advancements in continuous glucose sensor technology and development of algorithms that modulate insulin infusion rates based on glucose trends to target euglycemia. The pharmacokinetics of short acting insulin used in closed-loop systems are such that even with complete cessation of insulin infusion, there remains a depot of subcutaneous insulin that acts to lower glucose for 4-6 hours.[38] Including glucagon within these systems reduces frequency and duration of hypoglycemia[39-43] without requiring the patient to interact with the system or intake carbohydrates.

Glucagon offers a natural addition to these closed-loop systems. However, as with mini-dose glucagon, this will require a stable liquid formulation before this is feasible outside the research setting. Currently, the only commercially available closed-loop system, Medtronic MiniMed 670 g (Northridge, CA), is an insulin-only system. However, of the 18 or more closed-loop systems in development, there are at least 6 that are insulin and glucagon dual hormone systems; Inreda artificial pancreas, iLet Bionic Pancreas, Closed-Loop Assessment, Oregon bihormonal closed-loop system, bio-inspired artificial pancreas, IMA-AP.[44]

Problems to Overcome

The main challenge in developing a stable liquid glucagon is the predilection to form beta-pleated sheets of amyloid-like fibrils in aqueous solution. In fact, the normal storage of glucagon with the alpha cell vesicles is in amyloid-like fibrils.[45] There has been concern for the cytotoxicity of these fibrils, however in retrospect, this toxicity may have been due to the acidity and osmolality of the solutions on the cultured cells.[46-50] Packed fibrils of glucagon in aqueous solution leads to formation of firm gels[51] that will eventually clog a continuous infusion pump. In addition, glucagon spontaneously degrades through deamidation and oxidation in solution.[52,53] To date, research studies of dual hormone closed-loop systems have used lyophilized glucagon preparations that are typically reconstituted every 24 hours to fill the pump reservoirs.[39,40,54] In order to be used in closed-loop systems, glucagon will need to have stability in a liquid state at 37°C for at least 3-7 days. Viable products will likely need to have shelf life similar to insulin and better affordability than current glucagon rescue kits.[55]

Approaches to Stable Liquid Glucagon

Given the problems with native glucagon in aqueous solution, there are two main approaches to stable liquid glucagon that are described here: (1) native glucagon with special carrier solutions and (2) analogs of native glucagon that promote in solution stability.

Dasiglucagon (previously ZP4207) is in development by Zealand Pharma (Glostrup, Denmark).[56] This is an analog of human glucagon in which 7 of the 29 amino acids are substituted. Dasiglucagon is stable in aqueous solution with no reconstitution. Their website indicates plans to develop multiuse pen, pump and infusion formulations. Phase 2 trials showed comparable pharmacokinetic data to currently available lyophilized glucagon formulations. They showed a more robust and longer duration of hyperglycemic response in comparison to conventional glucagon at 1.0 mg dose.[57] To date, data suggest similar side effect profile to conventional glucagon with mild injection site reactions and dose dependent nausea and vomiting. Phase 3 trials are enrolling now to evaluate for efficacy, safety tolerability and immune response to repeated doses of Dasiglucagon in patients with type 1 diabetes.[58] Given this is a novel peptide; immunogenicity will likely need ongoing evaluation in human subjects.

Xeris (Austin, TX) is developing a liquid stable glucagon product based on their XeriSol platform.[59] The native human glucagon protein is dissolved in an aprotic polar solvent, dimethyl sulfoxide (DMSO), which is used in other FDA approved injectables.[60] Xeris's website indicates product path of multiuse pen, pump, and infusion formulations. Phase 2 trials show comparable pharmacokinetic/pharmacodynamics to current glucagon products, with slightly slower absorption at higher doses.[61,62] For the 2.0 μg/kg dose, the time to 50% absorption was delayed 2.9 minutes for the Xeris product compared to the currently available glucagon product.[62] Xeris has secured financing to move to phase 3 trials for their glucagon products.[63] There has been injection site discomfort with this product. Five of 16 patients reported significant enough discomfort to warrant not using the product in an ambulatory study of mini-dose glucagon.[35] However, within the extension portion of the study where

patients were given the option to use the Xeris glucagon or glucose tabs, 40% of hypoglycemia events were treated with the Xeris glucagon. It is unclear whether the patients who reported significant discomfort chose not to use the product in the extension study.[36]

Adocia (Lyon, France) is developing a BioChaperone form of native human glucagon that is an aqueous solution at neutral pH.[64] Their BioChaperone technology utilizes polymers, oligomers, and small organic compounds that form complexes with proteins to protect from degradation, and target specific absorption and duration of action profiles. The pharmacokinetic/pharmacodynamic data from animal studies show similar results to conventional glucagon.[64-66] They recently announced preliminary phase 1 human pharmacokinetic/pharmacodynamic results in patients with T1D indicating resolution of hypoglycemia within 11 minutes as compared to conventional glucagon rescue formulation resulting in resolution in 7 minutes.[64] Their website indicates product path to develop both hypoglycemia rescue and pump compatible products.

Several past endeavors to develop liquid stable glucagon are no longer being actively pursued. These include Biodel's (since acquired by Albireo Pharma, Boston, MA) formulation using lysolecithin as surfactant, a simple sugar and alcohol to form micelles to keep glucagon in solution.[25] Albireo is not pursuing further development of their glucagon products. (Personal communication) Liquid glucagon at an alkaline pH using ferulic acid to prevent degradation showed stability and animal pharmacokinetic/pharmacodynamic data similar to lyophilized glucagon; this is not actively being pursued.[67,68] Marcadia (purchased by Roche, Basel, Switzerland) appears to no longer working on their glucagon analogs MAR-D28 and MAR531. Latitude Pharma (San Diego, CA) is no longer actively developing Rescue G and PumpaGon native glucagon products for use in emergency rescue and pump applications respectively.[69] PumpaGon showed similar performance in animal pharmacokinetic/pharmacodynamics studies as fresh reconstituted lyophilized glucagon.[70] A combined effort from ProSciento (Chula Vista, CA) and Sanofi (Bridgewater, NJ), SAR438544, was presented at the 2017 77th ADA sessions.[71] This is a synthetic peptide agonist for the glucagon receptor that is based on the sequence of the Exendin-4 protein. Human pharmacokinetic/pharmacodynamic was somewhat disappointing showing a limited hyperglycemic effect as compared to conventional glucagon. Their study in patients with type 1 diabetes was closed prior to enrollment in September 2016.[72]

Conclusion

Hypoglycemia is a significant problem for health outcomes and quality of life in type 1 diabetes. Treatment of hypoglycemia has not changed in several decades and is limited to ingestion of carbohydrates for mild hypoglycemia or intramuscular injection for severe hypoglycemia. Currently available lyophilized forms of glucagon in emergency kits are difficult to use and error-prone in stressful situations. The option of intranasal glucagon will allow for robust glucose raising effects without the complexities of reconstitution and intramuscular injection allowing for successful use by untrained bystanders. Mini-dose glucagon is a new approach to the treatment of mild hypoglycemia, comprised of the use of low dose glucagon via the subcutaneous route. Initially developed for sick-day protocols in children; there is new evidence for equivalent efficacy to glucose tabs in adults. In addition, glucagon in dual hormone closed-loop systems reduces frequency and duration of hypoglycemia. However, all these new methods will require stable liquid glucagon formulations. The main problem to overcome with stable liquid glucagon is the tendency of native glucagon to fibrillate and form gels in aqueous solution. There are two main tactics to overcome this issue; native glucagon prepared with special carrier solutions and analogs of glucagon that promote in-solution stability. Several promising formulations are in the late stages of development. Future study will be needed to determine long-term safety and real-world efficacy of these new products. Meanwhile, patients and providers eagerly await the availability of these new glucagon products.

Abbreviations

DMSO, dimethyl sulfoxide; FDA, Food and Drug Administration.

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References

1. Unger RH, Orci L. Physiology and pathophysiology of glucagon. *Physiol Rev*. 1976;56:778-826.
2. Hoare SRJ. Mechanisms of peptide and nonpeptide ligand binding to Class B G-protein-coupled receptors. *Drug Discov Today*. 2005;10:417-427.

3. Chabenne JR, DiMarchi MA, Gelfanov VM, DiMarchi RD. Optimization of the native glucagon sequence for medicinal purposes. *J Diabetes Sci Technol*. 2010;4:1322-1331.
4. GlucaGen HypoKit. Prescribing information for GlucaGen HypoKit. Australia: Novo Nordisk Inc; 2016. Available at: http://www.novo-pi.com/glucagenhypokit.pdf. Accessed January 24, 2018.
5. Glucagon (rDNA Origin). Prescribing information for glucagon for injection (rDNA Origin). Indianapolis, IN: Eli Lilly and Company; 2017. Available at: http://pi.lilly.com/us/rglucagon-pi.pdf. Accessed January 24, 2018.
6. Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes*. 1991;40:223-226.
7. Dagogo-Jack SE, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. Recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. *J Clin Invest*. 1993;91:819-828.
8. Cryer PE. Diverse causes of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med*. 2004;350:2272-2279.
9. Taborsky GJ Jr, Ahrén B, Havel PJ. Autonomic mediation of glucagon secretion during hypoglycemia: implications for impaired alpha-cell responses in type 1 diabetes. *Diabetes*. 1998;47:995-1005.
10. Cryer PE, Davis SN, Shamoon H. Hypoglycemia in diabetes. *Diabetes Care*. 2003;26:1902-1912.
11. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. *Diabetes Care*. 1994;17:697-703.
12. Diabetes Control and Complications Trial Research Group; Nathan DM, Genuth S, Lachin J, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329:977-986.
13. Complications Trial Research Group. Hypoglycemia in the diabetes control and complications trial. *Diabetes*. 1997;46:271-286.
14. Miller KM, Foster NC, Beck RW, et al. Current state of type 1 diabetes treatment in the U.S.: updated data from the T1D Exchange clinic registry. *Diabetes Care*. 2015;38:971-978.
15. Weinstock RS, Xing D, Maahs DM, et al. Severe hypoglycemia and diabetic ketoacidosis in adults with type 1 diabetes: results from the T1D Exchange clinic registry. *J Clin Endocrinol Metab*. 2013;98:3411-3419.
16. Lonnen KF, Powell RJ, Taylor D, Shore AC, MacLeod KM. Road traffic accidents and diabetes: insulin use does not determine risk. *Diabet Med*. 2008;25:578-584.
17. Lapenta L, Di Bonaventura C, Fattouch J, et al. Focal epileptic seizure induced by transient hypoglycaemia in insulin-treated diabetes. *Epileptic Disord*. 2010;12:84-87.
18. Laing SP, Swerdlow AJ, Slater SD, et al. The British Diabetic Association Cohort Study, II: cause-specific mortality in patients with insulin-treated diabetes mellitus. *Diabet Med*. 1999;16:466-471.
19. Centers for Disease Control. National diabetes statistics report: estimates of diabetes and its burden in the United States. Centers for Disease Control and Prevention; 2017. Available at: https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf. Accessed September 15, 2017.
20. Snoek FJ, Hajos TR, Rondags S. Psychological effects of hypoglycaemia. In: Frier BM, Heller SR, McCrimmon RJ, eds. *Hypoglycaemia in Clinical Diabetes*. Oxford, UK: John Wiley; 2014:323-333.
21. Barendse S, Singh H, Frier BM, Speight J. The impact of hypoglycaemia on quality of life and related patient-reported outcomes in type 2 diabetes: a narrative review. *Diabet Med*. 2012;29:293-302.
22. Jönsson L, Bolinder B, Lundkvist J. Cost of hypoglycemia in patients with type 2 diabetes in Sweden. *Value Health*. 2006;9:193-198.
23. Fidler C, Elmelund Christensen T, Gillard S. Hypoglycemia: an overview of fear of hypoglycemia, quality-of-life, and impact on costs. *J Med Econ*. 2011;14:646-655.
24. Rombopoulos G, Hatzikou M, Latsou D, Yfantopoulos J. The prevalence of hypoglycemia and its impact on the quality of life (QoL) of type 2 diabetes mellitus patients (The HYPO Study). *Hormones*. 2013;12:550-558.
25. Steiner SS, Li M, Hauser R, Pohl R. Stabilized glucagon formulation for bihormonal pump use. *J Diabetes Sci Technol*. 2010;4:1332-1337.
26. Yale J-F, Dulude H, Egeth M, et al. Faster use and fewer failures with needle-free nasal gucagon versus injectable glucagon in severe hypoglycemia rescue: a simulation study. *Diabetes Technol Ther*. 2017;19:423-432.
27. Doe-Simkins M, Walley AY, Epstein A, Moyer P. Saved by the nose: bystander-administered intranasal naloxone hydrochloride for opioid overdose. *Am J Public Health*. 2009;99:788-791.
28. Pontiroli AE. Intranasal glucagon: a promising approach for treatment of severe hypoglycemia. *J Diabetes Sci Technol*. 2015;9:38-43.
29. Lilly's Clinical Development Pipeline. Clinical development pipeline. Indianapolis, IN: Eli Lilly; 2017. Available at: https://www.lilly.com/discovery/pipeline. Accessed January 24, 2018.
30. National Library of Medicine. Safety and efficacy of ZP-glucagon to injectable glucagon for hypoglycemia. ClinicalTrials.gov; 2015. Available at: https://clinicaltrials.gov/ct2/show/NCT02459938. Accessed September 15, 2017.
31. Haymond MW, Schreiner B. Mini-dose glucagon rescue for hypoglycemia in children with type 1 diabetes. *Diabetes Care*. 2001;24:643-645.
32. Brink S, Laffel L, Likitmaskul S, et al. Sick day management in children and adolescents with diabetes. *Pediatr Diabetes*. 2009;10(suppl 12):146-153.
33. Chung ST, Haymond MW. Minimizing morbidity of hypoglycemia in diabetes: a review of mini-dose glucagon. *J Diabetes Sci Technol*. 2015;9:44-51.
34. Hasan KS, Kabbani M. Mini-dose glucagon is effective at diabetes camp. *J Pediatr*. 2004;144:834.
35. Haymond MW, Redondo MJ, McKay S, et al. Nonaqueous, mini-dose glucagon for treatment of mild hypoglycemia in adults with type 1 diabetes: a dose-seeking study. *Diabetes Care*. 2016;39:465-468.
36. Haymond MW, DuBose SN, Rickels MR, et al. Efficacy and safety of mini-dose glucagon for treatment of nonsevere hypoglycemia in adults with type 1 diabetes. *J Clin Endocrinol Metab*. 2017;102:2994-3001.
37. Rickels MR, Dubose S, Wolpert H, et al. Mini-dose glucagon as a novel approach to prevent exercise-induced hypoglycemia in type 1 diabetes, 67-LB—2017. Poster presented at:

American Diabetes Association 77th Scientific Sessions; 2017; San Diego, CA. Available at: https://ada.scientificposters.com/epsAbstractADA.cfm?id=1. Accessed June 11, 2017.
38. Castle JR. Is glucagon needed in type 1 diabetes? *Lancet Diabetes Endocrinol*. 2015;3:578-579.
39. El-Khatib FH, Russell SJ, Nathan DM, Sutherlin RG, Damiano ER. A bihormonal closed-loop artificial pancreas for type 1 diabetes. *Sci Transl Med*. 2010;2:27ra27.
40. Castle JR, Engle JM, El Youssef J, et al. Novel use of glucagon in a closed-loop system for prevention of hypoglycemia in type 1 diabetes. *Diabetes Care*. 2010;33:1282-1287.
41. Haidar A, Legault L, Dallaire M, et al. Glucose-responsive insulin and glucagon delivery (dual-hormone artificial pancreas) in adults with type 1 diabetes: a randomized crossover controlled trial. *CMAJ*. 2013;185:297-305.
42. van Bon AC, Luijf YM, Koebrugge R, Koops R, Hoekstra JBL, DeVries JH. Feasibility of a portable bihormonal closed-loop system to control glucose excursions at home under free-living conditions for 48 hours. *Diabetes Technol Ther*. 2014;16:131-136.
43. Russell SJ, El-Khatib FH, Sinha M, et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N Engl J Med*. 2014;371:313-325.
44. Trevitt S, Simpson S, Wood A. Artificial pancreas device systems for the closed-loop control of type 1 diabetes. *J Diabetes Sci Technol*. 2015;10:714-723.
45. Maji SK, Perrin MH, Sawaya MR, et al. Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. *Science*. 2009;325:328-332.
46. Andersen CB, Otzen D, Christiansen G, Rischel C. Glucagon amyloid-like fibril morphology is selected via morphology-dependent growth inhibition. *Biochemistry*. 2007;46:7314-7324.
47. Beaven GH, Gratzer WB, Davies HG. Formation and structure of gels and fibrils from glucagon. *Eur J Biochem*. 1969;11:37-42.
48. Onoue S, Ohshima K, Debari K, et al. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. *Pharm Res*. 2004;21:1274-1283.
49. Pedersen JS. The nature of amyloid-like glucagon fibrils. *J Diabetes Sci Technol*. 2010;4:1357-1367.
50. Ward WK, Massoud RG, Szybala CJ, et al. In vitro and in vivo evaluation of native glucagon and glucagon analog (MAR-D28) during aging: lack of cytotoxicity and preservation of hyperglycemic effect. *J Diabetes Sci Technol*. 2010;4:1311-1321.
51. Jackson MA, Caputo N, Castle JR, David LL, Roberts CT Jr, Ward WK. Stable liquid glucagon formulations for rescue treatment and bi-hormonal closed-loop pancreas. *Curr Diab Rep*. 2012;12:705-710.
52. Joshi AB, Rus E, Kirsch LE. The degradation pathways of glucagon in acidic solutions. *Int J Pharm*. 2000;203:115-125.
53. Caputo N, Castle JR, Bergstrom CP, et al. Mechanisms of glucagon degradation at alkaline pH. *Peptides*. 2013;45:40-47.
54. Jacobs PG, El Youssef J, Reddy R, et al. Randomized trial of a dual-hormone artificial pancreas with dosing adjustment during exercise compared with no adjustment and sensor-augmented pump therapy. *Diabetes Obes Metab*. 2016;18:1110-1119.
55. Rylander D Jr. Glucagon in the artificial pancreas: supply and marketing challenges. *J Diabetes Sci Technol*. 2015;9:52-55.
56. Zealand Pharma. Zealand Pharma. Copenhagen, Denmark: ZEAL; 2017. Available at: https://www.zealandpharma.com/. Accessed October 9, 2017.
57. Hövelmann U, Bysted BV, Mouritzen U, Macchi F, Lamers D, Kronshage B, Møller DV, Heise T. Pharmacokinetic and Pharmacodynamic Characteristics of Dasiglucagon, a Novel Soluble and Stable Glucagon Analog. Diabetes Care Am Diabetes Assoc; 2017.
58. National Library of Medicine. A trial to evaluate the immunogenicity of dasiglucagon and glucagen in patients with type 1 diabetes mellitus. ClinicalTrials.gov; 2017. Available at: https://clinicaltrials.gov/ct2/show/NCT03216226?term=dasiglucagon&rank=2. Accessed September 15, 2017.
59. Xeris Pharma. Xeris pharmaceuticals. Austin: Xeris Pharmaceuticals; 2017. Available at: https://www.xerispharma.com. Accessed January 24, 2018.
60. McKim AS, Strub R. Dimethyl sulfoxide USP, PhEur in approved pharmaceutical products and medical devices. *Pharm Technol*. 2008;32:74.
61. Strange P, Cummins MJ, Prestrelski SJ, Shi L, Christiansen M. A pilot study in adults with T1DM to examine the efficacy of stable nonaqueous glucagon for treatment of severe hypoglycemia, 140-LB—2017. Poster presented at: American Diabetes Association 77th Scientific Sessions; 2017; San Diego, CA. Available at: https://ada.scientificposters.com/epsAbstractADA.cfm?id=4. Accessed June 11, 2017.
62. Castle JR, Youssef JE, Branigan D, et al. Comparative pharmacokinetic/pharmacodynamic study of liquid stable glucagon versus lyophilized glucagon in type 1 diabetes subjects. *J Diabetes Sci Technol*. 2016;10:1101-1107.
63. Xeris Pharma. Xeris pharmaceuticals announces closing of $30 million financing. GlobeNewswire News Room; 2017. Available at: https://globenewswire.com/news-release/2017/05/17/987533/0/en/Xeris-Pharmaceuticals-Announces-Closing-of-30-Million-Financing.html. Accessed September 15, 2017.
64. BioChaperone Glucagon. BioChaperone Human Glucagon. Lyon, France: Adocia; 2017. Available at: https://www.adocia.com/. Accessed December 11, 2017.
65. Meiffren G, Teng S, Ranson A, et al. Preclinical efficacy of a stable aqueous formulation of human glucagon with BioChaperone Technology (BC GLU), 1150-P—2017. Poster presented at: American Diabetes Association 77th Scientific Sessions; 2017; San Diego, CA. Available at: https://ada.scientificposters.com/epsAbstractADA.cfm?id=3. Accessed June 11, 2017.
66. Soula O, Duracher D, Budin G, et al. BioChaperone technology enables rhGlucagon aqueous formulation for use in rescue and dual-hormone artificial pancreas (DHAP). Poster presented at: American Diabetes Association 77th Scientific Sessions; 2017; San Diego, CA. Available at: https://ada.scientificposters.com/epsAbstractADA.cfm?id=4. Accessed June 11, 2017.
67. Ward K, Castle JR, Caputo N, Bakhtiani PA. Formulations comprising glucagon. US Patent, 20150291680A1. Portland: Oregon Health & Science University; 2015. Available at: https://patents.google.com/patent/US20150291680A1/en. Accessed August 22, 2017.

68. Bakhtiani PA, Caputo N, Castle JR, et al. A novel, stable, aqueous glucagon formulation using ferulic acid as an excipient. *J Diabetes Sci Technol*. 2015;9:17-23.
69. Latitude Pharma. Latitude pharma. San Diego: LATITUDE Pharmaceuticals Inc; 2003. Available at: http://latitudepharma.com/ Accessed January 24, 2018.
70. Castle JR. New drugs: glucagon. Presentation at: Annual Diabetes Technology Meeting; November 10-22, 2016; Bethesda, MD.
71. Hompesch M, Grosjean P, Morrow L, et al. The novel glucagon receptor agonist SAR438544, first in human safety, pharmacokinetic, and pharmacodynamic data from a study in healthy volunteers, 1066-P—2017. Poster presented at: American Diabetes Association 77th Scientific Sessions; 2017; San Diego, CA. Available at: https://ada.scientificposters.com/epsAbstractADA.cfm?id=1. Accessed June 11, 2017.
72. National Library of Medicine. A study to evaluate safety, pharmacokinetics, and pharmacodynamics of a single dose of SAR438544 in comparison to glucagon in type 1 diabetes mellitus patients under induced hypoglycemia. ClinicalTrials.gov; 2015. Available at: https://clinicaltrials.gov/ct2/show/NCT02635243. Accessed September 15, 2017.