REVIEW

New uses and formulations of glucagon for hypoglycaemia

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Abstract

Hypoglycaemia is the more frequent complication of insulin therapy and the main barrier to tight glycaemic control. Injectable glucagon and oral intake of carbohydrates are the recommended treatments for severe and non-severe hypoglycaemia episodes, respectively. Nasal glucagon is currently being developed as a ready-to-use device, to simplify severe hypoglycaemia rescue. Stable forms of liquid glucagon could open the field for different approaches for mild to moderate hypoglycaemia treatment, such as mini-doses of glucagon or continuous subcutaneous glucagon infusion as a part of dual-hormone closed-loop systems. Pharmaceutical companies are developing stable forms of native glucagon or glucagon analogues for that purpose.

Keywords: type 1 diabetes, glucagon, nasal glucagon, hypoglycaemia, dual-hormone artificial pancreas, exercise, mini-dose glucagon, injectable glucagon.

Introduction

Optimal glycaemic control is essential to avoid microvascular diabetes complications, but the main barrier to reaching normoglycaemia is the risk of hypoglycaemia.[1] Many recent advances in therapy for people with type 1 diabetes have been introduced in clinical practice, such as continuous subcutaneous insulin infusion, continuous glucose monitoring, sensor-augmented pumps, and fast-action insulin analogues. These treatments have been shown to improve glycaemic control in clinical trials[2] and real-world studies.[3,4] Despite all these advances, hypoglycaemia remains the most common complication of insulin therapy in people with diabetes.

The International Hypoglycaemia Study Group defines a hypoglycaemia event as clinically relevant, if blood glucose falls below 54 mg/dL (3 mmol/L).[5] Severe hypoglycaemia is defined as any episode of abnormally low blood glucose levels leading to cognitive impairment and consequently needing third-party assistance for its treatment.[6] Given that children have a limited capacity to detect and self-treat hypoglycaemia, the International Society of Pediatric and Adolescent Diabetes (ISPAD) defines severe hypoglycaemia in the paediatric population as an event associated with severe neuroglycopenic symptoms, frequently coma and convulsions, requiring parenteral treatment with glucose or glucagon.[7]

The frequency of hypoglycaemia in people with type 1 diabetes is estimated to be two events per week of non-severe hypoglycaemia and one episode of severe hypoglycaemia per year.[8] In the paediatric population, the estimated incidence of severe hypoglycaemia ranges between 5 and 20 per 100 patients per year.[9]

Exercise is a major challenge for people with diabetes. The increased glucose uptake by the muscles and the increased insulin sensitivity, which may persist for 8–24 hours after exercise, significantly increase the risk of hypoglycaemia.[10]

Hypoglycaemia has important physical and psychological consequences. Severe hypoglycaemia is a potentially life-threatening situation; it may lead to sudden death, presumably due to cardiac arrhythmia, and, if profound and prolonged, to cerebral death. Subsequently, the fear of hypoglycaemia may itself also become a barrier for achieving tight glycaemic control.[11,12]

Guidelines recommend the intake of oral carbohydrates for non-severe hypoglycaemia treatment and injectable glucagon for severe hypoglycaemia episodes, as the patient is unable to consume carbohydrates.[13]

The purpose of this review was to collect the most recent advances regarding new uses and new formulations of glucagon for hypoglycaemia treatment and prevention. We conducted a literature search, using the PubMed electronic

database, looking for papers published up to June 2019. We performed the search strategy using 'glucagon', 'nasal glucagon', 'dual-hormone artificial pancreas', 'mini-dose glucagon', and 'injectable glucagon' as keywords.

Glucagon physiology

In 1923, a component in pancreatic extracts was found to induce transient hyperglycaemia. This substance was named glucagon, to reflect its 'glucose agonist' effect.[14] Glucagon is a 29-amino-acid peptide biologically synthesised by pancreatic alpha cells.[15,16] It is considered a relevant counterregulatory hormone with a key role in the maintenance of normoglycaemia, as it counteracts the effect of insulin in glucose metabolism regulation.

Glucagon exerts its main action by stimulating hepatic glycogenolysis. It also increases gluconeogenesis, making glucose available for peripheral tissues when the exogenous supply is low.[17] Other known actions of glucagon are the induction of ketogenesis, appetite suppression, delayed gut motility, and also an effect in protein and lipid metabolism.[18] Recently, it has been found that glucagon might play a bigger role than previously thought in amino-acid metabolism.[19]

Glucagon for severe hypoglycaemia treatment

The only approved treatment for severe hypoglycaemia in the out-of-hospital setting is intramuscular glucagon (1 mg), as the patient is unable to take carbohydrates orally. This strategy has been used for more than four decades because it rapidly elevates blood glucose levels. The efficacy of intramuscular glucagon as a rescue for severe hypoglycaemia has been well established, and its failure rate has been proved to be low.[20]

Glucagon is unstable in liquid form, so it is presented as a lyophilized powder that needs to be reconstituted immediately before its use. Currently, the only commercially available formulations are GlucaGen Hypokit (Novo Nordisk, Copenhagen, Denmark) and Glucagon Emergency Kit (Eli Lilly, Indianapolis, IN).

In a situation of severe hypoglycaemia, intramuscular glucagon must be reconstituted and administered to the patient by a caregiver. This approach has a number of limitations and makes injectable glucagon challenging to use, as the reconstitution and administration of the preparation are complex, and several steps are required. When it has to be administered by a non-trained caregiver, in a non-medical environment and in a stressful situation, the administration may be delayed, and the number of handling errors is likely to be high. Several usability studies have shown that only 13% of caregivers delivered the full dose of injectable glucagon during a simulated severe hypoglycaemia episode.[21] In addition, due to this complexity in its preparation procedure, the injectable glucagon is recognized to be underprescribed and underused.[22]

Nasal glucagon for severe hypoglycaemia

A novel, needle-free, easy-to-use form of glucagon is desirable to overcome the mentioned limitations of parenteral glucagon for the treatment of severe hypoglycaemia.[23]

Nasal glucagon was first shown to be passively absorbed through the nasal mucosa and to increase blood glucose levels in healthy subjects in 1983.[24] Although small studies using intranasal glucagon solutions and intranasal glucagon powders also showed positive results in adults and children with type 1 diabetes, at that moment the pharmaceutical industry did not take the opportunity to develop this formulation.[23] Currently, a nasal formulation of glucagon is undergoing development.

This nasal glucagon is presented as a single-use, ready-to-use, portable device containing 3 mg of dry powder synthetic glucagon, identical to the human recombinant glucagon in emergency kits, allowing a simple one-step administration of nasal glucagon (Table 1).

Several studies have shown that a 3 mg dose of nasal glucagon is an effective and safe alternative to intramuscular glucagon in adults and children in clinical trials[25,26] and real-world outpatient settings.[27,28] In addition, the same dose could be used for children up to 4 years old.[23]

Nasal glucagon has to be administered by the caregiver into the patient´s nostril, as it is absorbed by the nasal mucosa. It has been shown that nasal congestion, common colds, or concomitant administration of nasal decongestants do not reduce the efficacy of nasal glucagon.[29] Patient inhalation, which could be impossible in a situation of impaired consciousness, is not needed.[26]

The use of nasal glucagon does not require reconstitution by the caregiver. In addition, the non-parenteral route of administration makes less probable the erroneous administration of insulin instead of glucagon. Nasal glucagon has been found to be easier to administer and preferred by non-trained caregivers rather than injectable glucagon.[28]

Nasal glucagon is well tolerated. No severe adverse effects have been reported with its use. The main reported adverse effects are nausea, vomiting and headaches, and local effects such as nasal discomfort or congestion.

This formulation of nasal glucagon was originally developed by Locemia Solutions, Montreal, Canada, in 2010, and has been manufactured by Eli Lilly, Indianapolis, Indiana, since 2016. Nasal glucagon is currently awaiting approval by the FDA and European Medicines Agency (EMA) as a rescue treatment for severe hypoglycaemia, and it is intended to be commercially available in the near future.

Finally, the use of nasal glucagon as a more effective treatment of severe hypoglycaemia could reduce the use of expensive professional emergency services and the number

Table 1. New glucagon formulations.

Glucagon formulation	Company	Indication	Dose	Route	Side effects	Phase trial
Nasal glucagon	Eli Lilly	Severe hypoglycaemia	3 mg	Nasal	Nausea, vomiting headaches nasal discomfort	Phase III
Xerisol glucagon	Xeris Pharmaceuticals	Mild-to-moderate hypoglycaemia Severe hypoglycaemia	0.5–1 mg	Subcutaneous	Nausea, vomiting headaches injection site reactions	Phase III
Dasiglucagon	Zeeland Pharma	Mild-to-moderate hypoglycaemia Severe hypoglycaemia	0.1–1 mg	Subcutaneous	Nausea, vomiting headaches injection site reactions	Phase III
BioChaperone glucagon	Adocia	Mild-to-moderate hypoglycaemia Severe hypoglycaemia	0.5–1 mg	Subcutaneous	Nausea, vomiting headaches possible local reactions	Phase I

of hospital admissions, reducing the economic burden of hypoglycaemia.[30]

Glucagon for mild and moderate hypoglycaemia treatment

For non-severe hypoglycaemia, the recommended treatment is the intake of 15–20 g of oral carbohydrates. This approach is simple and effective, but it increases the number of calories ingested by the patients. Type 1 diabetes patients suffer an average of two episodes of non-severe hypoglycaemia per week, meaning that they need an extra 120 kcal per week, 60 kcal for each hypoglycaemia episode.[8] The percentage of type 1 diabetes patients who are overweight or obese is rapidly increasing, so the extra calories consumed for hypoglycaemia treatment are considered a disadvantage.[31]

In addition, the oral intake of carbohydrates frequently causes a rebound hyperglycaemia due to overtreatment by the patient. This hyperglycaemia requires correction insulin, leading to a vicious circle of unstable diabetes control.

Exercise is a situation of increased risk of hypoglycaemia and can be challenging for people with type 1 diabetes. Exercise-induced hypoglycaemia can be a barrier to exercise practice. The recommended amount of carbohydrates to avoid hypoglycaemia during exercise is as high as 30–60 g/hour of exercise. The ingestion of such a large amount of carbohydrates can be undesirable from a weight-control perspective and also unpleasant.

An alternative treatment for mild to moderate hypoglycaemia would be desirable. In addition, having a better option to carbohydrate intake to treat exercise-induced hypoglycaemia could help patients engage in exercise.

Subcutaneous mini-dose glucagon

The use of low doses of glucagon has been proposed as a way to prevent or treat mild to moderate hypoglycaemia in children and adults.[32] Low doses of glucagon have been demonstrated to increase plasma glucose after insulin infusion-induced hypoglycaemia.[33]

Low subcutaneous doses of glucagon, also known as glucagon mini-doses, have been used off-label for the management of sick days in type 1 diabetes children, when they are unable or unwilling to eat, such during a gastroenteritis episode.[34] Current ISPAD guidelines propose the use of small doses of glucagon for sick days management as a way to prevent hospital admissions in children with type 1 diabetes.[35]

Doses of 150 µg of glucagon have also been shown to be effective for preventing or treating mild to moderate hypoglycaemia in adults,[36,37] minimizing the need for additional calorie intake and helping to control body weight.

The injection of 150 µg of glucagon may be more effective than insulin reduction and cause less hyperglycaemia after exercise than the oral intake of glucose tablets.[38] The glucose response to low-dose glucagon is not impaired by moderate exercise.[39]

It has been proposed that a bolus calculator could be designed to help patients decide the dose of glucagon they need to use depending on their insulin on board when facing a hypoglycaemia episode in an open-loop setting.[40]

Glucagon in closed-loop systems

Due to the relatively slow onset and long duration of current insulin formulations, even after the introduction of fast-acting insulin analogues, the achievement of tight glycaemic control

through automated insulin infusion in closed-loop systems is challenging.

Multiple research groups are developing systems that automatically deliver glucagon and insulin, known as dual-hormone artificial pancreas systems.[41–47]

These dual-hormone closed-loop systems infuse intermittent microboluses of glucagon in response to sensor data showing imminent hypoglycaemia. In these systems, glucagon is used to treat or prevent mild hypoglycaemia, so smaller doses than those used for severe hypoglycaemia treatment will be required. Differences in the infusion rates of glucagon with the different algorithms have been found, with an estimated dose of 0.033–0.82 mg/day.[41,48] These differences could have an impact on the risks to benefit balance of glucagon use in these systems, so it needs to be investigated in long-term trials.

The dual-hormone approach reduces the time spent in hypoglycaemia and possibly has an added benefit in overall glycaemic control in comparison to insulin-only artificial pancreas systems.[48,49] The possibility of more aggressive glucose targets could achieve a more strict glycaemic control, and the reduced need for carbohydrate intake could lead to greater satisfaction for the patient.[50]

On the other hand, it must be recognized that adding glucagon to a closed-loop system would increase its complexity. A need for a dual reservoir and infusion system would increase the burden for the patient. In addition, a more complex algorithm to control the system would be necessary. Recently, a coordinated control algorithm for insulin and glucagon has been proposed.[51] The use of glucagon would also increase the cost of the therapy. In addition, there is concern about the effectiveness of glucagon in dual-hormone systems after ethanol intake,[52] a low carbohydrate diet,[53] or with concomitant high insulin infusion.[54] Further research is needed to help clinicians decide which patients would benefit most from these dual-hormone systems.

Alternatively, an intraperitoneal route for continuous infusion of glucagon has been proposed to obtain a faster glucose response.[55]

Glucagon in congenital hyperinsulinism

Congenital hyperinsulinism is the most frequent cause of persistent hypoglycaemia in the neonatal period and early childhood, ranging between 1:20,000 and 1:50,000 of live births.[56,57]

Congenital hyperinsulinism is characterized by uncontrolled insulin secretion. Delayed treatment can lead to severe hypoglycaemia and an increased risk of convulsions, impaired neuromotor development, and permanent cerebral damage. Focal congenital hyperinsulinism is an indication for partial

pancreatectomy, which completely cures the disease. Diffuse congenital hyperinsulinism is first approached by medical treatment, such as diazoxide and the somatostatin analogue, octreotide.[58] In recent years, new pharmacologic therapies such as long-action somatostatin analogues or immunosuppressant agents such as sirolimus have been tried.[59,60] Only if this approach fails, a near-total pancreatectomy is required.[61,62] Despite many diagnostic and therapeutic advances, congenital hyperinsulinism remains a significant cause of morbidity in children, and 26–44% of children with this disorder have permanent intellectual disabilities, particularly when it was diagnosed in the neonatal period.

A therapeutic alternative for congenital hyperinsulinism treatment would be continuous subcutaneous glucagon infusion, at a dose of 0.026–0.8 mg/kg/day. This therapy has been successfully used in children not responding to diazoxide, allowing a reduction or withdrawal of glucose infusion. In patients treated with octreotide, the octreotide dose was notably reduced, avoiding a pancreatectomy.[63] In a more recent study, continuous subcutaneous glucagon infusion has allowed restoration of normoglycaemia, mitigating the weight increase and improving the development in a patient with diffuse congenital hyperinsulinism.[64]

Stable formulations of glucagon could make continuous subcutaneous glucagon infusion an alternative for the treatment of this rare disease.

Stable forms of glucagon

The currently commercially available formulations of glucagon are highly unstable in liquid form.[65] Glucagon tends to form fibrils shortly after reconstitution in aqueous solution, causing loss of activity and also leading to an increased risk of occluding pump infusion catheters. In addition, these aggregates could be potentially cytotoxic at high concentrations.

For that reason, glucagon is provided as lyophilized powders, and it must be reconstituted before use, used immediately after reconstitution, and discarded after use. This presentation, although suitable for severe hypoglycaemia rescue, is not suitable for the development of new indications, such as the use of low doses of glucagon for mild to moderate hypoglycaemia treatment or for use in artificial pancreas systems.[66]

Currently, for dual-hormone closed-loop studies, glucagon has been diluted to provide continuous subcutaneous infusion. However, the infusion set needs to be changed every 24 hours, which is safe but not appropriate for real-world use.[67] Stable glucagon is necessary to enable the commercialization of automated glucagon delivery closed-loop systems, as these systems would need stable glucagon for at least 3 days at 37°C. The use of low doses of glucagon for non-severe hypoglycaemia prevention or treatment also requires stable glucagon.[68] In addition, stable glucagon would make possible

a presentation of glucagon in a pen as an option for severe hypoglycaemia treatment.

Aware of this unmet need in hypoglycaemia treatment, pharmaceutical companies are developing different soluble glucagon preparations for glucagon injections. There are two possible approaches in the search for stable glucagon: native glucagon in different formulations to make it stable, or new stable glucagon analogues (Table 1). Furthermore, the development of microneedle transdermal patch delivery of native glucagon has been recently reported.[69]

Stable native glucagon

A native form of stable liquid glucagon[36,37,66,70] is under development by Xeris Pharmaceuticals (Austin, TX, USA). Based on their XeriSol™ platform,[71] this room-temperature-stable non-aqueous liquid form of glucagon is intended for subcutaneous injection with a reusable pen (G-Pen™ Glucagon and G-Pen Mini™ Glucagon) and for pump infusion (G Pump™ Glucagon). In Xerisol glucagon, the native human glucagon protein is dissolved in an aprotic polar solvent, dimethyl-sulfoxide (DMSO), and mild injection site reactions, such as discomfort and burning, have been reported.[68,70]

Dasiglucagon

Dasiglucagon, previously ZP4207, is a novel analogue of glucagon that is stable in aqueous formulation and does not need reconstitution.[72,73] In a dasiglucagon molecule, 7 out of 29 amino acids are substituted to obtain a formulation stable in liquid form. Currently, dasiglucagon is being developed by Zeeland Pharma AS (Copenhagen, Denmark).

Dasiglucagon has pharmacokinetic and pharmacodynamic profiles that are similar to lyophilized formulations and rapidly increases plasma glucose in a dose-dependent manner.[74] Recently, dasiglucagon has been tried in a feasibility study delivered by a dual artificial pancreas system.[75] Currently, dasiglucagon in the treatment of postprandial hypoglycaemia after Roux-en-Y gastric bypass is being tested.

As dasiglucagon is a novel synthetic peptide, its safety profile must be closely monitored, regarding local reactions, immunogenicity, and possible long-term adverse effects. So far, no immunogenic side effects have been shown.

BioChaperone glucagon

Adocia (Lyon, France) is developing a BioChaperone form of native human glucagon. The Biochaperone technology prevents glucagon from degrading by adding compounds that form complexes with proteins.[68,76,77] In this manner, adding BioChaperone makes glucagon stable. Short-term and long-term side effects of BioChaperone glucagon have to be evaluated.

Limitations of the use of glucagon

The risks involved in the use of glucagon have to be considered. The short-term side effects of glucagon, such as nausea, vomiting, and headaches, are well known, and the use of low doses of glucagon would minimize these side effects. However, the possible long-term consequences of chronic use of glucagon are still unknown. Possible gastrointestinal, cardiovascular, or central nervous system side effects have to be further evaluated, for both native glucagon and glucagon analogues, given the multisystemic actions of glucagon.[41] A rare but possible long-term side effect of chronic use of glucagon is necrolytic migratory erythema.

The cost of the use of the glucagon as an alternative treatment for hypoglycaemia needs to be evaluated. The glucagon required per day for non-severe hypoglycaemia prevention or treatment in dual-hormone artificial pancreas systems can reach 0.8 mg/day. In addition, an analysis of manufacturing, supply, and marketing needs to be carried out.[78]

Conclusion

Hypoglycaemia is a common and clinically relevant complication of insulin therapy in people with diabetes when trying to achieve optimal glycaemic control. Oral intake of carbohydrates and parenteral glucagon are the only available approaches for mild-to-moderate and severe hypoglycaemia episodes, respectively. These approaches have not changed in decades. There is an unmet need for alternative forms and routes for delivery of glucagon for the treatment and prevention of hypoglycaemia episodes. Although this hormone was discovered a long time ago, recently there has been a renewed interest in glucagon and in the search for stable glucagon, mainly linked to the development of closed-loop systems infusing both insulin and glucagon. Companies are currently developing stable glucagon liquid formulations.

New formulations of glucagon, such as nasal glucagon or stable preparations of glucagon for subcutaneous injection or continuous subcutaneous infusion in artificial pancreas systems, could help to treat or prevent hypoglycaemia in a broad spectrum of situations. Nasal glucagon is a promising alternative to currently available injectable glucagon that would substantially change the treatment of severe hypoglycaemia. Mini-dose glucagon is an effective therapeutic tool to prevent or treat mild to moderate hypoglycaemia and could become a non-caloric choice to treat mild hypoglycaemia.

New formulations of glucagon would simplify and extend glucagon use in children and adults, reducing the burden of living with diabetes.

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