



Antibodies in metabolic diseases

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In the past century, incidences of chronic metabolic diseases, such as obesity and type II diabetes, have increased dramatically. Obesity and abnormal insulin level are associated with a wide variety of health problems including a markedly increased risk for type II diabetes, fatty liver, hepato-biliary and gallbladder diseases, cardiovascular pathologies, neurodegenerative disorders, asthma and a variety of cancers.

The development of therapeutic antibodies has evolved over the past decades into a mainstay of therapeutic options for patients with inflammatory diseases and cancer, while other indication areas such as metabolic diseases have so far only been rarely addressed.

Although therapeutic antibodies might have advantages over current type II diabetes treatments like favorable serum half-life and high specificity, their development is also likely to face obstacles. For example the technical feasibility of antibody generation against G protein coupled receptors and transporters is challenging, patient compliance for a likely needle application might be limited, bioavailability in organs involved in the pathogenesis like the brain might be suboptimal and reimbursement issues for high treatment costs have to be taken into account.

The current review focuses on the pathogenesis and standard therapeutic approaches as well as antibodies in development and potential antibody targets for type II diabetes.

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Introduction

The incidence of chronic metabolic diseases, particularly obesity and type II diabetes, has increased dramatically [1] in adults, adolescent and even more remarkably in children [2]. Despite continuing to be a disease of the elderly population (adult onset diabetes) the age of onset of type II diabetes continuously lowers, which creates an increasing need for novel, improved therapies.

Obesity, hypertension, hypertriglyceridemia and hyperinsulinemia are the key factors of the metabolic syndrome also referred to as “deadly quartet”. The metabolic syndrome has reached epidemic levels worldwide. An analysis performed in 2009 revealed that more than one third of the adult population in the US above the age of 20 meets the criteria for metabolic syndrome [3]. The worldwide market size of metabolic syndrome treatments was estimated at US \$ 97.7 Billion in 2008. Addressing

the central mechanisms underlying these pathologies should lead to new therapeutic approaches for treating these conditions.

Numerous medications are routinely used to treat patients with metabolic syndrome putting them at risk for the occurrence of drug–drug interactions and adverse reactions (“side effects”). Although there are various available oral therapies which control glucose levels, most patients develop an insulin-dependent diabetes during their lifetime. These issues may be circumvented by specifically targeting a common molecule that modulates multiple pathways of the syndrome.

Despite a high medical need for improved therapies, the development of therapeutic antibodies in metabolic diseases is limited and often addresses the inflammatory part of the pathogenesis only.

The two most advanced monoclonal antibodies in development for the treatment of type II diabetes are canakinumab (Ilaris[®],

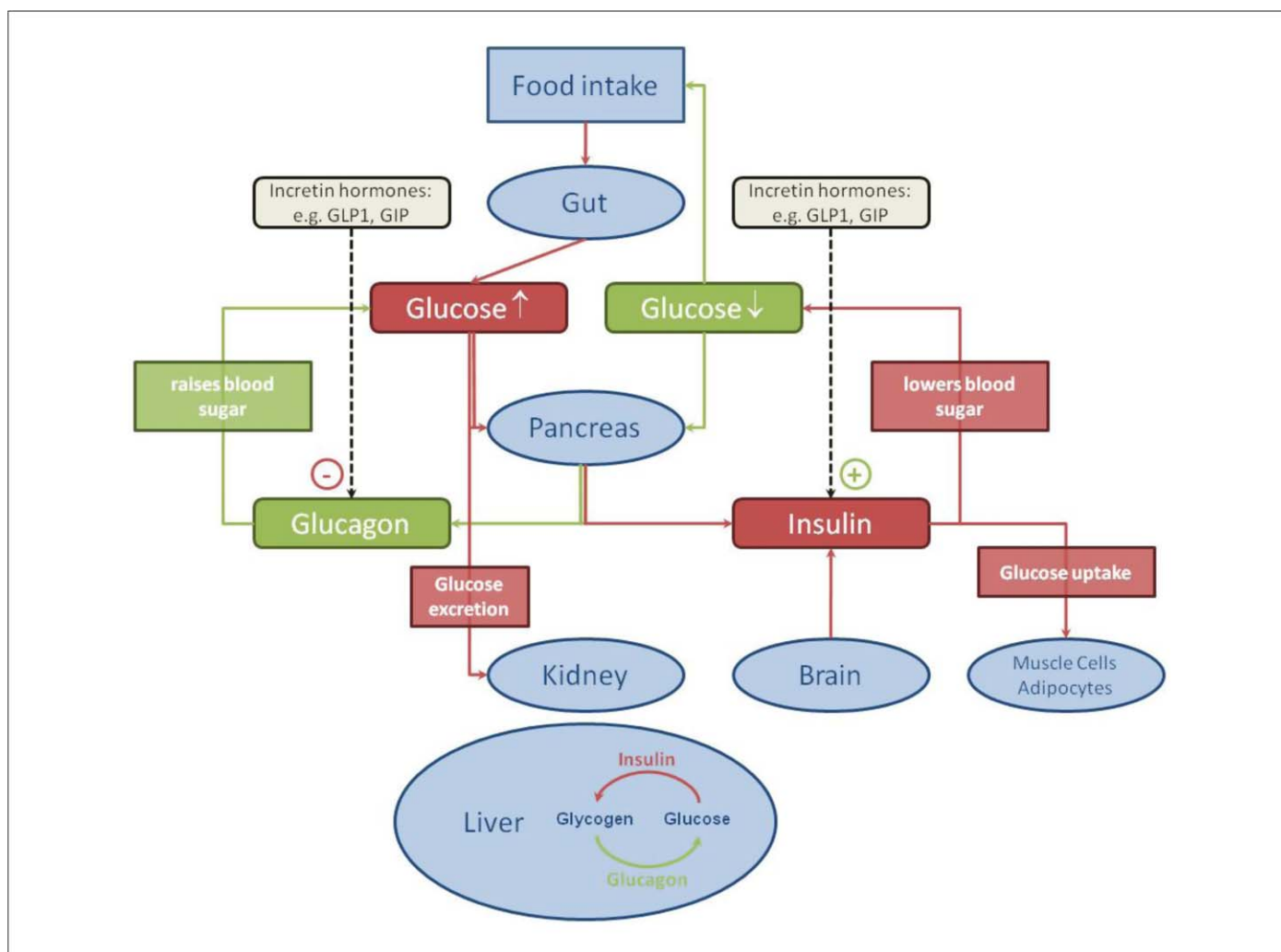


FIGURE 1

Blood glucose control by hormones: following entry of food into the gut glucagon-like peptide 1 (GLP1) is released into the circulation. Blood glucose and GLP1 stimulate insulin and inhibit glucagon secretion from pancreatic islets which raises the circulating insulin and lowers the secretion of glucose by the liver. In addition insulin augments glucose uptake in muscle cells and adipocytes. Meal ingestion stimulates signals through the brain which trigger insulin release as well.

Novartis; in phase II) and gevokizumab (XOMA052, XOMA; in phase II). Both are human anti-interleukin-1 (IL-1) beta monoclonal antibodies designed to alleviate inflammatory symptoms of type II diabetic patients without treating the underlying issue, the insulin resistance.

This review briefly introduces the pathogenesis, addresses limits of standard therapeutic approaches, highlights antibodies in development and future challenges for the treatment of type II diabetes. Although not all potential antibody targets for type II diabetes can be covered by this review a selection of the most effective and promising targets and potential reasons why there are to date not more antibodies in development will be addressed in the following.

Physiology and pathogenesis

Blood glucose homeostasis is controlled by endocrine cells of the pancreas which are located in the islets of Langerhans. The islet cells monitor the concentration of glucose in the blood and secrete hormones with opposite effects. Insulin is produced and released by pancreatic beta-cells and lowers circulating glucose, whereas glucagon is produced and released by pancreatic alpha cells and raises circulating blood glucose.

The system of hormones, which control glucose homeostasis is even more versatile. Following entry of food into the gut, incretin hormones (e.g. glucagon like peptide (GLP-1), gastric inhibitory peptide (GIP)) are released into the circulation. Blood glucose and incretin hormones stimulate insulin release and inhibit glucagon secretion from pancreatic islet cells, which (1) lowers the circulating glucose, (2) augments glucose uptake into muscle cells and adipocytes, (3) stimulates signals to the brain which trigger insulin release and (4) accelerate renal excretion of glucose (Fig. 1).

Islet dysfunction characterized by a combination of impaired insulin secretion, inappropriately high glucagon secretion and reduced beta-cell mass has a central role in type II diabetes. Very important is the need for the beta-cell mass to be closely regulated by glucose and hormonal effects on beta-cell replication, size, apoptotic elimination and under certain conditions, neogenesis from progenitor cells. Failure to adapt to changes in body mass, pregnancy, insulin sensitivity of peripheral tissues, or tissue injury may lead to the development of chronically elevated blood glucose, or diabetes [4,5].

Besides dysfunction of pancreatic islet cells, also the failure of body cells and tissues to respond to secreted insulin can lead to hyperglycemia. The so-called insulin resistance can be caused by e.g. reduced insulin receptor density, insulin receptor dysfunction or neutralizing auto-antibodies to insulin. The resulting hyperglycaemia can facilitate beta-cell failure in the pancreas and worsen insulin resistance, thus triggering a cycle of impaired metabolism and glycototoxicity [6].

Obesity and type II diabetes are closely linked. Most patients suffering from type II diabetes present with co-morbid overweight or obesity [7]. Chronic obesity potentially leads to increased insulin resistance and type II diabetes which is probably due to adipose tissue being a source of several hormone and cytokine signals to other tissues. Others have shown that type II diabetes causes obesity as an effect of the changes in metabolism and other deranged cell behavior attendant on insulin resistance [8] (Fig. 2).

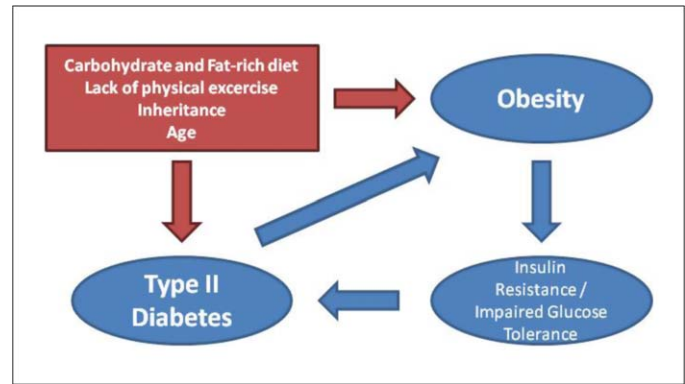


FIGURE 2

Type II diabetes and obesity are closely linked and both can cause or at least increase the probability of developing each other. Chronic obesity leads to increased insulin resistance. Type II diabetes changes the metabolism leading to obesity and further anti-diabetics can induce weight gain.

Standard therapeutic approaches

The basis for the treatment of type II diabetes is improving the glycaemic control. This can initially be achieved by lifestyle changes like increased physical activity and dietary modifications with the objective to reduce body weight. If these activities are no longer sufficient pharmacological intervention can be supportive. Pharmacologic therapies include oral therapeutics like biguanides, sulphonylureas, thiazolidinediones, meglitinides, alpha-glucosidase inhibitors, incretin analogues, DPP4 inhibitors as well as needle applications such as insulin substitution [9–13]. Several type II diabetes patients develop insulin dependence during their lifetime. Objective of pharmacological intervention is lowering blood glucose levels by various mechanisms. The following drugs are used as single agents and combination treatments.

- (1) Biguanides improve hyperglycaemic control primarily through suppression of hepatic glucose production and increase the glucose uptake by muscle cells and adipocytes and thus lowering the blood glucose level.
- (2) Sulfonylureas stimulate the insulin production in the pancreas.
- (3) Thiazolidinediones increase the glucose uptake by muscle cells, adipocytes and the liver in the presence of insulin.
- (4) Meglitinides stimulate insulin production in the pancreas in response to blood glucose levels.
- (5) Incretin analogues mimic the function of GLP-1 whereby DPP4 inhibitors prevent the degradation of GLP-1.
- (6) Alpha-glucosidase inhibitors decrease the absorption of carbohydrates from the digestive tract to lower post-meal glucose levels.

Major challenges in type II diabetes therapy are that in spite of aggressive therapy glycaemic control may still worsen over time. Current therapy is frequently associated with hypoglycemia and weight gain and adverse events like e.g. gastrointestinal discomfort, edema or cardiac failure occur often during treatment. Thus, novel therapies that control hyperglycemia without inducing adverse events are highly desired.

Promising strategies, which could lead to the improvement of type II diabetes therapy are the increase of glucose cellular uptake, increase of glucose metabolism, suppression of glucagon response,

the increase of hepatic glucose uptake and the elimination of excess glucose. Novel therapies should be long acting and prevent or prolong the time to disease progression especially in younger patients.

There are various, novel and also well-known (potential) molecular targets, which could be either inhibited or stimulated to normalize blood glucose levels for the treatment of type II diabetes.

Therapeutic antibodies in development

Anti-Interleukin 1 (IL-1)/IL-1 receptor

Inadequate glucose control in type II diabetes is associated with systemic up-regulation of inflammatory markers [14]. Chronic hyperglycemia and inflammatory cytokines disrupt and/or attenuate signal transduction pathways that promote normal pancreatic beta-cell turnover and survival, leading to the destruction of the endocrine pancreas in type I and type II diabetes [15,16]. In contrast to the above-described pharmacologic interventions, which lower blood glucose level by various mechanisms, the antibodies against IL-1 are designed to alleviate inflammatory symptoms of type II diabetes. IL-1 is a pro-inflammatory cytokine, its tissue-destructive role has been described for several diseases [17]. Prolonged exposure of a pancreatic beta-cell line to increased glucose concentrations resulted in significant inhibition of insulin signaling, enhanced endogenous IL-1 beta expression and increased susceptibility of cells to apoptosis [18]. Recent studies from animal models suggest a causative role of IL-1 beta in the loss

of beta-cell mass [19]. Xoma and Novartis are developing the anti-IL-1 beta antibodies gevokizumab and canakinumab, respectively. Both antibodies are being clinically tested apart from other indications also in patients with type II diabetes.

Canakinumab is a fully human monoclonal anti-human IL-1 beta antibody of the IgG1/ κ isotype. Canakinumab binds with high affinity specifically to human IL-1 beta and neutralizes the biological activity of human IL-1 beta by blocking its interaction with IL-1 receptors, thereby preventing IL-1 beta-induced gene activation and the production of inflammatory mediators (EPAR product information [20]). Canakinumab was FDA and EMEA approved for the treatment of cryopyrin-associated periodic syndromes (CAPS) in 2009 and is currently tested in a phase II/III trial for type II diabetes with regulatory filings expected in 2012 (NCT00900146) and three additional type II diabetes studies (NCT01068860, NCT00995930 and NCT00605475).

Gevokizumab is a fully human monoclonal anti-human IL-1 beta antibody of the IgG2 isotype. Gevokizumab is a potent inhibitor of IL-1 beta activity that reduces the affinity of IL-1 beta for its signaling receptor and co-receptor but not for its decoy and soluble inhibitory receptors. This mechanism shifts the effective dose response of the cytokine so that the potency of IL-1 beta bound by gevokizumab is 20–100-fold lower than that of IL-1 beta in the absence of antibody in a variety of *in vitro* cell-based assays [21]. Gevokizumab is currently in two phase II clinical trials in type II diabetes patients (NCT01066715 and NCT01144975).

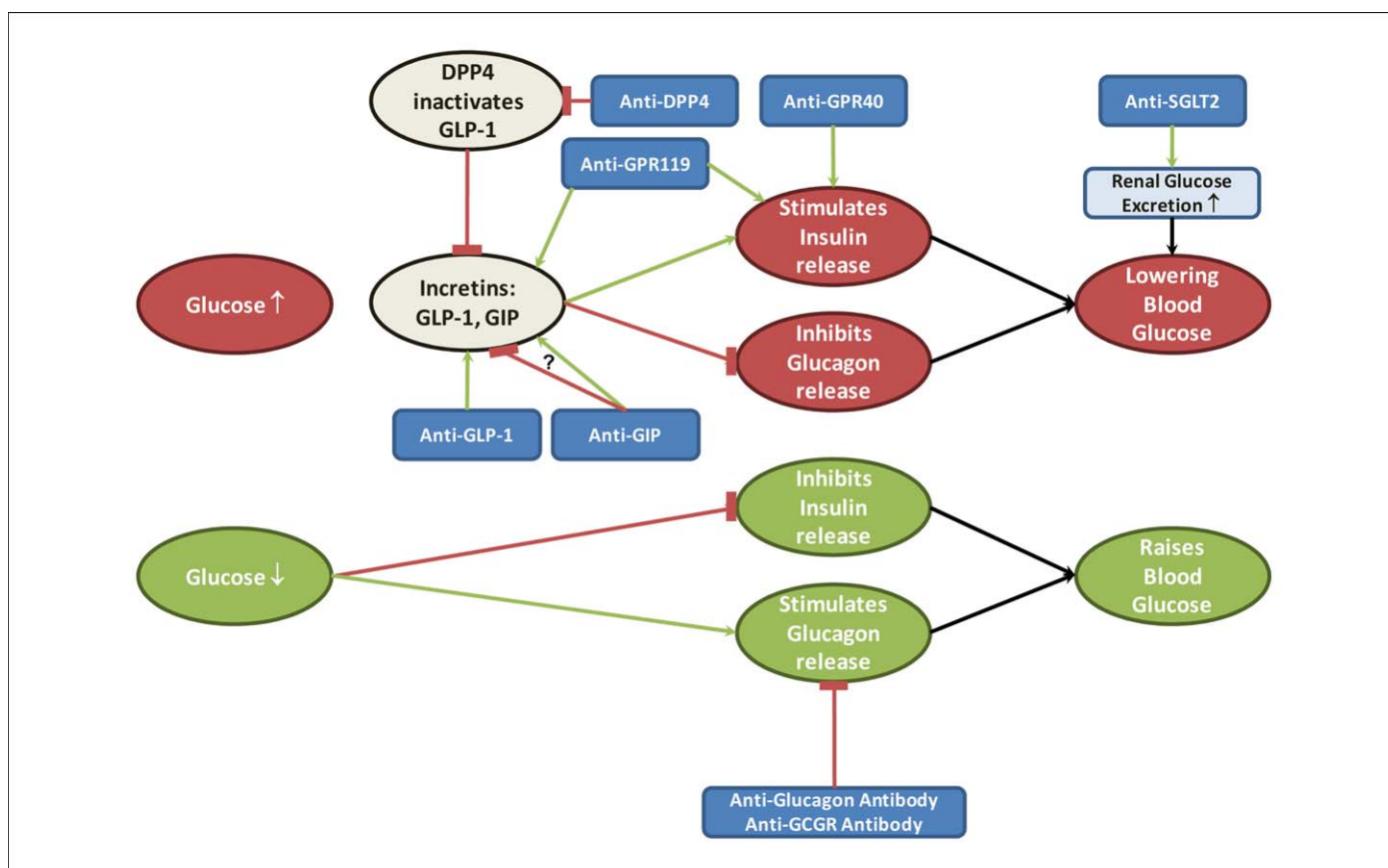


FIGURE 3

Various potential targets and points of interference to normalize blood glucose levels in type II diabetes. Inhibitory/antagonistic activities are depicted as red bars, activating/agonistic activities are depicted as green arrows. See text for details.

Development of AMG 108 (Amgen, human IgG1), another antibody targeting the same pathway via blockade of the IL-1 receptor rather than the ligand, was already in phase II for type II diabetes but was withdrawn (NCT00661921) and development in this indication was discontinued. Despite that, development in rheumatoid arthritis is ongoing (NCT00293826). It might be that beta-cells which express the IL-1 receptor are destroyed by antibody-dependent-cell-mediated functions, which would act in contrast to inhibiting the pro-apoptotic signaling of IL-1 beta. Thus inhibiting the ligand might be a more reasonable approach.

Potential antibody targets

Figure 3 gives an overview about possible intervention points which could potentially be addressed with therapeutic antibodies.

Glucagon and glucagon receptor (GCGR)

Glucagon is a peptide which is secreted in response to hypoglycemia by pancreatic alpha cells. Glucagon acts as an opponent of insulin, stimulating hepatic glucose production and thus increasing blood glucose [22,23]. Because glucagon stimulates hepatic glucose production, elevated glucagon levels may contribute to hyperglycemia in type II diabetes [24–26] and animal studies have shown that inhibition of glucagon signaling has glucose lowering effects [27–30]. After binding to the glucagon receptor, glucagon activates adenylyl cyclase in the hepatocyte plasma membrane and induces glycogenolysis via a cAMP-related signaling pathway. In addition, glucagon activates multiple enzymes required for gluconeogenesis including the enzyme system which converts pyruvate to phosphoenolpyruvate, the rate-limiting step in gluconeogenesis [31,32]. Studies characterizing glucagon receptor deficient mice or using glucagon receptor antagonists have further suggested that inhibition of glucagon binding to its receptor could potentially be an effective approach for improving glycaemic control in diabetes [33].

High-affinity glucagon neutralizing antibodies can effectively reduce free glucagon and glycemia in animal models [27,34,35]. It is therefore reasonable that glucagon neutralizing antibodies may prove valuable as therapeutic agents for the treatment of type II diabetes; however, daily injections of high doses of antibodies were required in animal models [36].

Targeting the glucagon receptor (GCGR) with antibodies is another possibility of inhibiting glucagon signaling which is an approach followed by Amgen (AMG 477). They developed fully human monoclonal antibodies which bind with higher affinity to GCGR than the natural ligand and elicit a highly specific antagonistic activity without inhibiting the closely related GLP-1 receptor. Despite rising glucagon levels, treatment with neutralizing mAbs maintained glucose-lowering efficacy [37]. Amgen was developing AMG 477 in phase I clinical development in the US. However, the antibody no longer appears in the Amgen pipeline. Development may have been discontinued for unknown reasons.

Targeting glucagon and/or glucagon receptor is appealing for several reasons as discussed above. Potential concerns regarding such an approach include that it might result in hypoglycemia although GCGR deficient mice have lower blood glucose level but are not hypoglycaemic [38]. This suggests a compensatory mechanism and it remains to be seen if antagonists will trigger similar compensation. In addition, it remains to be investigated if

GCGR antagonists will result in unfavorable accumulation of lipids in the liver because it is known that glucagon reduces lipogenesis by multiple mechanisms [33].

Glucagon-like peptide 1 (GLP-1)

GLP-1 receptor activation via GLP-1 mimetics was identified as potential therapeutic approach in 1992 [39] and introduced as a clinical therapy in 2005. Natural GLP-1 is secreted from endocrine L-cells within the gastrointestinal mucosa. It stimulates insulin secretion and inhibits glucagon secretion [40]. Active GLP-1 has a plasma-half life of only 1–2 min due to the rapid inactivation by DPP4 [41]. Half-life as well as exposure time has been increased after the identification of a GLP-1 analogue, which was isolated from the Gila monster and named exendin-4. Exendin-4 shows 53 percent homology to GLP-1 and binds to the GLP-1 receptor with similar affinity as GLP-1 but is resistant to DPP4 inactivation. Exendin-4 half-life in human is approximately 30 min after intravenous administration and two to three hours after subcutaneous administration [42]. A downside of exenatide (Byetta[®]; Amylin/Eli Lilly, synthetic form of exendin-4) is the induction of antibody responses in approximately 40 percent of patients. Most responses are low-titer responses with no clinical consequences; however, a subset of patients develop high-titer responses [43,44]. The consequences are not analyzed in detail; however, such responses might reduce drug efficacy as well. GLP-1 mimetics primarily target the islet dysfunction in type II diabetes. GLP-1 receptors are additionally expressed in other tissues such as the brain, heart, kidney and lung. It needs to be further investigated, which effects such extra-islet tissue expression has on the treatment [42]. Several GLP-1 analogues are in late-stage clinical development [42].

An antibody as GLP-1 mimetic might have a different availability to extra-islet tissues and would have the potential of longer half-life and reduced immunogenicity than currently available GLP-1 mimetics. Apart from its peripheral effects on potentiating the glucose-dependent insulin secretion, trophic and proliferative effects on pancreatic beta-cells, GLP-1 was also described to inhibit appetite via binding to central nervous system (CNS) GLP-1 receptors as well as through delayed gastric emptying which are both considered to contribute to weight loss [45]. Pfizer develops CVX096 (PF 4856883), a long-acting GLP-1 mimetic CovX-Body for subcutaneous administration for the treatment of type II diabetes in phase I clinical development (Pfizer Pipeline January 27, 2010). A CovX-Body is a peptide and antibody fusion protein which is bivalent and extends peptide actions through prolonged half-life.

Dipeptidylpeptidase 4 (DPP4)

Inhibiting the enzyme DPP4 prevents the inactivation of GLP-1 and is an alternative strategy to stimulate the GLP-1 receptor. DPP4 inhibitors such as Sitagliptin (Januvia[®], Merck) and Vildagliptin (Galvus[®], Novartis) are orally available, safe and well tolerated with a low risk of adverse events such as hypoglycaemia [46]. In contrast to GLP-1 mimetics they do not seem to have effects on weight [42].

DPP4 is a domain of the CD26 molecule, which consists of the DPP4 domain, a fibronectin and adenosine deaminase binding domain and a highly glycosylated membrane proximal region. A

humanized anti-CD26 therapeutic antibody, which is in clinical phase I/II, development for mesothelioma, has been developed by Y's Therapeutics. It is unclear which CD26 domain is targeted by this antibody and whether it has therapeutic potential for type II diabetes (R&D insight).

Gastric inhibitory peptide (GIP)

GIP, also known as glucose-dependent insulinotropic polypeptide, has recently been postulated to link over-nutrition with obesity. GIP is expressed by duodenal and jejunal K cells as well as by adipocytes and facilitates the degradation of both glucose and fat [47]. Similar to GLP-1, GIP is an incretin hormone, which stimulates the release of insulin [48] and beta-cell proliferation [49]. In contrast to GLP-1, GIP stimulates glucagon secretion under euglycaemic conditions [50] and GIP-induced insulin secretion is reduced in type II diabetes [51]. This might be due to the down-regulation of GIP receptors which is supported by a rat diabetes model in which the islet GIP receptor density was reduced. Reduction of hyperglycaemia in such diabetic rats restores the expression of GIP receptors [52]. Taking this into account, GIP receptor activation could be a potential therapeutic strategy after an initial treatment of the hyperglycaemia [42].

In contrast to the paragraph above, GIP receptor-deficient mice were completely protected from diet-induced obesity [53]. Thus, disrupting GIP signaling might represent a promising novel therapeutic strategy for the treatment of obesity. Indeed, a GIP-receptor antagonist led to reduced weight gain in mice fed a high-fat diet and weight loss in obese mice [54–56]. Vaccination against GIP results in high level of specific antibodies and prevents excessive body weight gain in rodents fed a high-fat diet and induces increased body weight loss in obese mice [57,58]. Despite influencing the body weight by active immunization, passive immunization under the tested conditions was not associated with any changes in body weight, food intake or metabolic control [58]. Reasons were not further investigated however, the treatment scheme might not have been sufficient to induce a sustained GIP knockdown [58]. Currently there are no further reports on the development of therapeutic antibodies acting as GIP antagonists.

Literature supports a rationale for both agonists and antagonist; it clearly needs further target validation to prove whether GIP targeting would be beneficial for the treatment of type II diabetes.

G protein-coupled receptor 40 (GPR40)

GPR40 is a G protein-coupled receptor (GPCR) which is activated by medium- to long chain free fatty acids, is predominantly expressed in pancreatic beta-cells and augments glucose-stimulated insulin secretion. In lower levels the receptor is also found in the brain, adipocytes, enteroendocrine cells and in pancreatic alpha cells [59–61].

Down-modulation of GPR40 by small interfering RNA or antisense oligonucleotides inhibits fatty acid induced insulin secretion by pancreatic beta-cells [62,63]. GPR40 deficient mice have a defective incretin and insulin response to free fatty acids [60,64–66]. In addition, a loss-of-function mutation of the GPR40 gene, which exists in 0.75 percent of healthy subjects was identified to be associated with obesity and impaired insulin secretion [67].

Several small molecule GPR40 agonists have been developed that show insulin secretion in a glucose-dependent manner and reduction of blood glucose levels in mice [68]. Thus, GPR40 is a promising cell surface target for the generation of agonistic antibodies. A Japanese group already developed a monoclonal antibody targeting GPR40 [69]. It remains to be demonstrated whether such antibodies can influence GPR40 functions.

G protein-coupled receptor 119 (GPR119)

GPR119 is a GPCR which is activated by lipid amides [70]. GPR119 expression is largely restricted to insulin-producing beta-cells of pancreatic islets and L-cells of the gastrointestinal tract. There was also expression in the brain reported [70,71]. Stimulation of GPR119 under hyperglycaemic conditions leads to a dual nutrient dependent insulinotropic and incretinotropic effect to maintain glucose homeostasis [70,72].

GPR119 mRNA levels were elevated in islets of obese hyperglycaemic db/db mice as compared to control islets, suggesting a possible involvement of this receptor in the development of obesity and diabetes [73]. Down-modulation of GPR119 by small interfering RNA inhibits insulin secretion in the rat pancreas and in a mouse beta-cell line [72]. In contrast to these findings, GPR119 deficient mice were normal and retained a normal insulin secretory response to glucose and GLP-1 [71]. Currently only early stage development for several small molecule GPR119 agonists has been reported [74–76]. The most advanced molecule is MBX-2982 (Metabolex) which was recently partnered with Sanofi-Aventis and is expected to complete phase II clinical trial end of 2010. Three other molecules are in different stages of clinical development (GSK-1292263, GlaxoSmithKline, phase II; PSN-821, OSI/Prosition, phase II; APD-597, Ortho-McNeil/Arena, phase I).

Sodium-glucose co-transporter 2 (SGLT2)

SGLT2 is a sodium glucose co-transporter, which is almost exclusively expressed in the kidney and is involved in glucose reabsorption. SGLT2 has been proposed as therapeutic target for diabetes [77]. The kidney has a key role in regulating glucose levels by mediating the reabsorption of glucose back into the plasma following filtration of the blood. Patients with diabetes have an increased capacity for renal glucose reabsorption [78]. Inhibition of SGLT2 elevates the renal excretion of glucose and thus lowers plasma glucose levels. In contrast to the other described approaches SGLT2 inhibition does not target one of the major physiological defects in type II diabetes (insulin resistance and impaired insulin secretion) however, is considered as promising treatment option. So far no data on anti-SGLT2 antibody approaches have been reported.

Conclusion

Increasing incidence and prevalence of type II diabetes in combination with limited efficacy of and adverse reactions of currently available therapies, stress the need for the development of novel therapies. Especially the increased incidence in children and adolescents prolongs the treatment duration and alter requirements for type II diabetes therapy. The generation of therapeutic antibodies would address limitations of small molecule therapies, such as therapeutic antibodies which confer higher specificity and target selectivity than small molecule inhibitors. Therapeutic

antibodies also have a significantly longer serum half-life than peptides and small molecules and potentially reduced side effects in comparison to current treatments. However, compared to therapeutic antibodies for the treatment of cancer and inflammatory diseases, with already 10 and 13 FDA approved therapeutic antibodies on the market, development of monoclonal antibodies for the treatment of metabolic disease is still in a very early stage. Possible cause for this could be the technical feasibility of generating antibodies against “challenging” antigen molecules such as GPCRs and transporters, which usually only have small extracellular domains which are further masked by glycosylation. In addition, patient compliance for likely needle applications might be limited; bioavailability in organs involved in the pathogenesis

like the brain could be sub-optimal. The major challenge in developing new type II diabetes therapies is the prevention of long-term consequences, such as cardiovascular disease, chronic renal failure and retinal damage. Long-term efficacy may be difficult to demonstrate in short clinical trials; although weight reduction and sufficient glucose control are regarded as valuable surrogates. It remains to be demonstrated that payers would reimburse expensive treatments without the demonstration of prevention of long-term consequences or considerable prolongation of the time until such long time consequences occur.

Conflict of interest

The author declares to be employed by MorphoSys AG.

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