The Dual Amylin- and Calcitonin-Receptor Agonist KBP-042 Works as Adjunct to Metformin on Fasting Hyperglycemia and HbA1c in a Rat Model of Type 2 Diabetes

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ABSTRACT
KBP-042 is a dual amylin and calcitonin receptor agonist that increases glucose tolerance and insulin action and reduces body weight in rat models of obesity and prediabetes. The objective of the present study was to 1) evaluate KBP-042 as a treatment of late-stage type 2 diabetes in a rat model and 2) assess the value of adding KBP-042 to the standard of care, metformin, to consider KBP-042 as a relevant drug for treating patients with type 2 diabetes. Two studies were included: an intervention study and a prevention study. In the intervention study, treatment with 5 mg/kg KBP-042 was initiated in 11-week-old Zucker diabetic fatty (ZDF) rats, in which glucose tolerance, fasting glycemia, and glycated hemoglobin were assessed after 4 weeks. In the prevention study, either metformin (400 mg/kg), KBP-042 (5 μg/kg), or a combination of both were administered to ZDF rats for a total of 9 weeks. Glycemia, glucose tolerance, and insulin tolerance were tested. Furthermore, fasting plasma insulin and glucagon levels were evaluated. Finally, pancreatic content of insulin was assessed as a surrogate marker of beta-cell mass. It was found that KBP-042 was efficient in lowering fasting plasma glucose as well as improving glucose tolerance, both as prevention and intervention of disease progression. Furthermore, KBP-042 was efficient in combination with metformin and had additional effects compared with either therapy alone. In conclusion, KBP-042 is a highly relevant therapeutic candidate against type 2 diabetes, effective both as an add-on therapy to metformin and as a stand-alone therapy.

Introduction
Metformin is the standard of care for type 2 diabetes; however, due to lack of efficacy metformin regularly fails to maintain glucose control after progression of the disease (Pawlyk et al., 2014). Consequently, add-on therapy is required for continued glucose control, which should preferentially work in combination with metformin, i.e., by targeting disease aspects not regulated by this, such as glucagon hypersecretion and excessive body weight. Alternatively, improving insulin sensitivity could provide the opportunity for synergistic effects with metformin (Hundal et al., 2000; Gong et al., 2012; Pawlyk et al., 2014). However, at the present time no treatment (including stand-alone and combination therapies) has shown the ability to increase insulin sensitivity and glycemic control while also reducing body weight (Kahn et al., 2006; Haas et al., 2014; Scott, 2014; Scheen, 2015).

The amylin analog pramlintide does not have the capacity to improve glycemic control alone, only as an adjunct therapy to insulin. It does so by lowering postprandial hyperglycemia through suppression of glucagon secretion, slowing of gastric emptying, increasing satiation, and facilitating weight loss (Ryan et al., 2009; Duncan et al., 2010; Traina and Kane, 2011). Generally, use of pramlintide is limited by its low potency; hence, more potent amylin analogs are of substantial interest since their mode of action clearly complements that of metformin (Mack et al., 2010, 2011).

Recent studies of an orally delivered novel dual amylin- and calcitonin-receptor agonist (DACRA), KBP-042, demonstrated markedly improved efficacy compared with the natural ligands, amylin and calcitonin. Oral KBP-042 improved glucose homeostasis in Zucker diabetic fatty (ZDF) rats by reducing fasting plasma glucose, glycated hemoglobin A1c (HbA1c) levels, ameliorating pancreatic dysfunction, and increasing insulin sensitivity. These effects have not been observed with known amylin analogs (Ryan et al., 2009; Duncan et al., 2010; Traina and Kane, 2011). When tested for the previously reported parameters for salmon calcitonin (another DACRA), KBP-042 showed superior efficacy and is thus promising as a novel treatment of type 2 diabetes (Feigh et al., 2011, 2012; Andreassen et al., 2014; Hjuler et al., 2015). All of the previous studies dealt with prevention of symptoms (obesity, hyperglycemia, insulin resistance) rather than initiation of treatment in a model that has progressed into a late stage of disease. Furthermore, the effects of subcutaneous delivery of KBP-042 on fasting plasma glucose and HbA1c have not been assessed. Finally, KBP-042 has only been evaluated as a monotherapy; however, it is relevant to evaluate the additive effects with metformin since most type 2 diabetic

ABBREVIATIONS: DACRA, dual amylin- and calcitonin-receptor agonist; HbA1c, glycated hemoglobin A1c; OGTT, oral glucose tolerance test; ZDF, Zucker diabetic fatty.
patients receive metformin due to its status as a first line of
treatment as recommended by the American Diabetes Association
and European Association for the Study of Diabetes (Thomas
and Gregg, 2017). In this study, we used ZDF rats to investigate the
efficacy of the injected KBP-042 in combination with the
standard of care high-dose metformin to assess the treatment
potential in a model that suffers from obesity as well as hyper-
glycemia, the two hallmarks of human type 2 diabetes.

Materials and Methods

Animal Experiments

All animal procedures were performed in accordance with guide-
lines from the Animal Welfare Division of the Danish Ministry of
Justice under the institutional license issued to Nordic Bioscience
(2012-15-2934-00094). Male obese ZDF rats (fa/−fa) (Charles River
Laboratories, Lyon, France) were obtained at 6 weeks of age and
housed pairwise under controlled temperature on a normal 12-hour
light-dark cycle with unrestricted access to water and food in a
standard type IV cage. The ZDF rats were fed Purina Laboratory Diet
#5008 (Brogaarden, Lyng, Denmark) and allowed 1 week of acclima-
tization prior to experiments. Two studies were performed in ZDF
rats, and in both studies the treatment groups were matched for body
weight, HbA1c, and fasting blood glucose levels. For subcutaneous
delivery the peptide KBP-042 (Andreassen et al., 2014) (Senn
Chemicals, Dielsdorf, Switzerland) was dissolved in sterile saline.

Animal Study 1: 4-Week Intervention Study with KBP-042.

The 11-week-old ZDF rats (kept on a regular diet to allow full
development of diabetes) were assigned vehicle treatment (n = 5)
and treatment with KBP-042 (n = 5). The vehicle group received s.c.
saline and the treatment group received 5 μg/kg/d KBP-042 (1 ml/kg)
dissolved in saline for 4 weeks. Fasting blood glucose (6 hours) was
measured weekly, and HbA1c was measured at the end of each study.
An oral glucose tolerance test (OGTT) at 1 g/kg was performed on day
20 of KBP-042 treatment in animals fasted for 12 hours. Animals
were administered with vehicle or drug at t = −30 and glucose was
administered at t = 0. Blood samples were collected just before drug
administration and glucose administration, and then following 15, 30,
60, and 120 minutes. At the end of the 4 weeks of treatment, animals
were fasted for 12 hours, blood was sampled for insulin measure-
ments, and animals were euthanized by exsanguinations under
isoflurane anesthesia.

Animal Study 2: 9-Week Combination Therapy with
KBP-042 and Metformin. Forty 7-week old ZDF rats were assigned
to the following groups: 1) vehicle; 2) s.c. KBP-042 5 μg/kg/d (1 ml/kg);
3) 400 mg/kg/d metformin by mouth (5 ml/kg); and 4) s.c. KBP-042
5 μg/kg/d in combination with 400 mg/kg/d oral metformin. The
vehicle group received saline (s. c. ml/kg) and by mouth (5 ml/kg),
likewise the s.c. group received saline by mouth and the group receiv-
ing oral metformin received s.c. saline.

Baseline blood samples were drawn on day 1 after 5 hours fasting
for measurements of HbA1c, fasting insulin, and glucagon. The
animals were administered with drugs for 9 weeks. Fasted (6 hours)
and nonfasted plasma glucose levels were measured on alternating
weeks while HbA1c was measured at the end of the study. In-
traperitoneal insulin tolerance testing was performed after 6 hour
fasting (insulin: 1 U/kg, humulin; Eli Lilly, Indianapolis, Indiana) at
week 7 of the study. The OGTT was also performed after 8 weeks of
treatment, as described previously. After 9 weeks animals were fasted
for 5 hours, blood was sampled for insulin and glucagon measure-
ments, and animals were euthanized by exsanguinations under
isoflurane anesthesia.

Biochemical Analysis. Blood samples were collected from the
tail vein of conscious animals in MiniCollect 1 ml EDTA tubes, and
centrifuged at 5000 rpm for 10 minutes at 4°C and kept at −20°C until

Results

Animal Study 1: 4-Week Intervention Study with
KBP-042. The ZDF rats were left untreated until 11 weeks of
age, where fasting blood glucose levels had increased to an
average of 28 mM. After development of hyperglycemia, treatment
with KBP-042 was initiated. KBP-042 treatment resulted in an immediate reduction in fasting glucose levels,
which were sustained throughout the study period (Fig. 1A).
Furthermore, the HbA1c levels at the end of the study were
reduced from 9.0% to 7.2% in the KBP-042-treated group
(Fig. 1B). An OGTT performed at week 8 (Fig. 1, C and D)
demonstrated a significant increase in glucose tolerance in
response to KBP-042. Notably, the increased glucose tolerance
was achieved with lower plasma insulin levels in the KBP-042
group compared with vehicle (Fig. 1, E and F). Finally, the
intervention with KBP-042 did not lead to significant change
in fasting plasma insulin levels at the end of the 4-week study
(Fig. 1G).

Animal Study 2: 9-Week Combination Therapy with
KBP-042 and Metformin. The effects of combined treat-
ment with KBP-042 and metformin were investigated in ZDF
rats. Treatment with either KBP-042 or metformin led to
increases in body weight, and the combination led to an even
larger weight gain (Fig. 2A). Treatment with metformin led to
lowering of both fasting and nonfasting blood glucose values in
the early stages of the study, which were not maintained at
later stages of the study (Fig. 2, B and C). Meanwhile, KBP-
042 induced a marked lowering of both fasting and nonfasting
blood glucose levels throughout the entire study (Fig. 2, B and
C). For the combination treatment, considerable decreases in
both glucose parameters were observed, indicating the addi-
tive effects of the two treatments (Fig. 2, B and C). Similarly,
the HbA1c levels in all treatment groups were lowered, and
the combination treatment was clearly superior to either
treatment alone with a 5% HbA1c reduction (Fig. 2D). Fasting
plasma insulin and glucagon were assessed both at baseline
and after treatment. Insulin levels in the vehicle group were
significantly lowered throughout the study (Fig. 2E), corre-
sponding to a loss of beta cells seen in the ZDF rat and leading
to drastically increased glucagon levels in the vehicle group.
The drastic increase in plasma glucagon was neutralized by
KBP-042 treatment and even further reduced with the combination of KBP-042 and metformin (Fig. 2F).

In the OGTT, both treatments led to an increase in glucose tolerance; however, the kinetics of blood glucose shown in Fig. 3A was distinct between the two treatment types. None of the treatment groups had a large increase in plasma glucose in response to oral glucose load; however, the basal glucose levels of the KBP-042 groups (both monotherapy and combination therapy) were lower than metformin and the vehicle group. This difference in basal levels is reflected in the area under the curve. Importantly, combination therapy was superior to either therapy alone (Fig. 3B). Both treatments increased insulin action, which was assessed by use of an intraperitoneal insulin tolerance test (Fig. 3E). The combination therapy had additive effects to the two treatments, and led to a large reduction in the
The preserving effect on beta cells seen after treatment with a DACRA (Feigh et al., 2012) was estimated by extracting insulin from the pancreases.
from animal studies 1 and 2. After the intervention study, where animals were left untreated first before initiation of treatment, no difference in insulin content in the pancreas was found (Fig. 4A). However, the combination treatment with KBP-042 and metformin administered throughout the study period did preserve the insulin content (Fig. 4B), suggesting that beta cells were preserved in contrast to the intervention study where the insulin content was low in both groups.

![Figure 3](image_url)

**Fig. 3.** KBP-042 in combination with high-dose metformin leads to superior glucose tolerance and insulin action in ZDF rats. (A) Kinetics of blood glucose changes during the OGTT performed in week 8. (B) Total area under the curve (AUC) of glucose during the OGTT. (C) Plasma insulin levels after glucose administration in the OGTT. (D) Total AUC of plasma insulin during the OGTT. (E) Kinetics of blood glucose during the intraperitoneal insulin tolerance test (IPITT) performed in week 7. (F) Total AUC of blood glucose during the IPITT. n = 10 rats per group. Statistical analyses between groups were performed as one-way analysis of variance followed by Tukey’s post hoc test with the following annotations: **P < 0.01, ***P < 0.001 versus vehicle; ††P < 0.01, †††P < 0.001 versus KBP-042 monotreatment; and ‡‡P < 0.01, ‡‡‡P < 0.001 versus metformin monotreatment. Data are expressed as mean ± S.E.M.
Discussion

DACRAs are agonists that are superior to both amylin and calcitonin on their respective receptors (Christopoulos et al., 1999; Andreasen et al., 2014). As treatment candidates, they separate themselves from amylin since they possess glucoregulatory capacities independent of insulin coadministration (Feigh et al., 2012; Andreasen et al., 2014). Recent studies have highlighted DACRAs as potential treatments for type 2 diabetes due to their ability to reduce fasting blood glucose, HbA1c, and body weight, and to increase insulin action (Feigh et al., 2011, 2012; Andreasen et al., 2014; Hjuler et al., 2016), warranting further analysis of their therapeutic potential in humans and possible combination strategies with existing medications. The main findings of the current studies are the following: 1) KBP-042 possesses potent glucoregulatory capacities even in severely diabetic animals, and 2) combining KBP-042 with metformin leads to additive improvements in glucose control compared with either monotherapy alone.

Administration of KBP-042 provided more robust pharmacodynamic effects than previously published using oral delivery (Andreasen et al., 2014), likely due to more homogenous exposure and subsequent biologic efficacy (Karsdal et al., 2015). Furthermore, KBP-042 has effects on fasting plasma glucose and insulin sensitivity that is not seen with amylin agonists such as pramlintide, which is possibly why pramlintide is only approved in combination with meal-time insulin (Ryan et al., 2009).

When KBP-042 was administered as an adjunct to high-dose metformin the combination was superior to either drug alone, demonstrating an additive effect. Since a large proportion of type 2 diabetic patients are on high-dose metformin as the first line of therapy, the present data suggest that KBP-042 could serve as a novel second line of therapy leading to improvements in glucose homeostasis, insulin tolerance, and body weight. Considering that metformin is known to work primarily by reducing excessive glucose production in the liver (Hundal and Inzucchi, 2003), the additive nature of the combination therapy may be expected, since the modes of action are distinct. The combination study, e.g., showed that when levels of fasting insulin were significantly reduced glucagon levels were increased inappropriately, probably due to the insufficient paracrine effect of insulin (Lee et al., 2012; Unger and Roth, 2015). This was efficiently ameliorated by treatment with KBP-042, and can possibly be attributed to preservation of beta cells (increased paracrine effect); however, amylin agonism also has a potent glucagonostatic effect (Gedulin et al., 1997; Akesson et al., 2003).

Another effect mediated by KBP-042 is reduction of gastric emptying (Hjuler et al., 2015). Gastric motility is often increased in type 2 diabetes (Frank et al., 1995; Horowitz et al., 2002), and slowing of the accelerated passing is known to reduce postprandial glucose, which is important in maintaining blood glucose homeostasis (Phillips et al., 2015). This, too, is an additive effect to that of metformin. The fasting insulin levels in the intervention study show that in this model the insulin-producing capacity has already been lost within the period without treatment. This was further corroborated by the unchanged pancreatic content of insulin after the study. However, the OGTT clearly demonstrates that KBP-042 treatment, even when introduced at a severely progressed stage, is able to increase glucose tolerance without increasing insulin levels. In fact, insulin levels were lower after KBP-042 administration. However, in the preventive setup in study 2 it was indicated that the combination treatment with KBP-042 and metformin is able to preserve beta cells and thereby maintain a sufficient high level of insulin. This finding was also confirmed by the increased insulin content in pancreas after treatment with metformin and KBP-042. Evidence that metformin improves insulin sensitivity also exists, and when considering that KBP-042 acts by increasing insulin action (Hjuler et al., 2016) the reason for potential synergy may reside at this level (Hundal et al., 2000; Gong et al., 2012; Pawlyk et al., 2014). Importantly, the increased insulin action is most likely the key determinant not only for the efficacy of

![Fig. 4](https://example.com/image.png)

**Fig. 4.** Pancreatic insulin content from extraction after treatment with (A) KBP-042 intervention for 4 weeks (after development of overt diabetes); n = 5 per group. Statistical analysis was performed as a Student’s t test; n.s., not significant. (B) Metformin, KBP-042, or a combination of metformin and KBP-042 for 8 weeks; n = 10 per group. Statistical analyses between groups were performed as one-way analysis of variance followed by Tukey’s post hoc test with the following annotations: ***P < 0.001 versus vehicle; ††P < 0.1 versus KBP-042 monotreatment; and †††P < 0.001 versus metformin monotreatment. Data are expressed as mean ± S.E.M.
KBP-042 but also for the additive effect observed in the combination therapy group. KBP-042 assists in rebalancing leptin levels, also potentially contributing to the synergy (Andreasen et al., 2014; Hjuler et al., 2015), as well as to a wide range of other metabolic effects (Unger and Roth, 2015).

An increase in body weight was observed after KBP-042 treatment in the ZDF rats, which is contradictory to the expected reduction in body weight. However, this weight gain is also seen with metformin, and it is most likely related to the improved health status of the treated animals when compared to the vehicle group. The data correlate well with previous findings in ZDF rats treated with other types of molecules, such as glucagon-like peptide 1 analogs and metformin, as well as previous DACRA studies, where treatment induces weight gain or rather prevents disease-related weight loss (Feigh et al., 2012; Vrang et al., 2012; Ito et al., 2013; Andreasen et al., 2014). In alignment, data from diet-induced obese rats have clearly demonstrated a weight-reducing effect with KBP-042 (Andreasen et al., 2014; Hjuler et al., 2015), underscoring that the lack of weight reduction is a phenomenon seen in ZDF rats only. In summary, KBP-042 possesses antidiabetic efficacy, which when combined with metformin leads to a potent treatment of severe hyperglycemia in a rat model of type 2 diabetes.

**Authorship Contributions**

*Participated in research design:* Hjuler, Henriksen, Karsdal.

*Conducted experiments:* Hjuler, Gydesen, Andreasen.

*Performed data analysis:* Hjuler, Gydesen, Andreasen.

*Wrote or contributed to the writing of the manuscript:* Hjuler, Henriksen.

**References**


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