AZD7545, a novel inhibitor of pyruvate dehydrogenase kinase 2 (PDHK2), activates pyruvate dehydrogenase in vivo and improves blood glucose control in obese (fa/fa) Zucker rats

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Abstract
PDH (pyruvate dehydrogenase) is a key enzyme controlling the rate of glucose oxidation, and the availability of gluconeogenic precursors. Activation of PDH in skeletal muscle and liver may increase glucose uptake and reduce glucose production. This study describes the properties of AZD7545, a novel, small-molecule inhibitor of PDHK (PDH kinase). In the presence of PDHK2, AZD7545 increased PDH activity with an EC₅₀ value of 5.2 nM. In rat hepatocytes, the rate of pyruvate oxidation was stimulated 2-fold (EC₅₀ 105 nM). A single dose of AZD7545 to Wistar rats increased the proportion of liver PDH in its active, dephosphorylated form in a dose-related manner from 24.7 to 70.3% at 30 mg/kg; and in skeletal muscle from 21.1 to 53.3%. A single dose of 10 mg/kg also significantly elevated muscle PDH activity in obese Zucker (fa/fa) rats. Obese, insulin-resistant, Zucker rats show elevated postprandial glucose levels compared with their lean counterparts (8.7 versus 6.1 mM at 12 weeks old). AZD7545 (10 mg/kg) twice daily for 7 days markedly improved the 24-h glucose profile, by eliminating the postprandial elevation in blood glucose. These results suggest that PDHK inhibitors may be beneficial agents for improving glucose control in the treatment of type 2 diabetes.

Introduction
PDH (pyruvate dehydrogenase) plays a pivotal role in controlling the balance between glucose and fatty acid oxidation, and its activation state is tightly controlled by the balance between specific PDHK (PDH kinase) and PDP (PDH phosphatase) activities. In type 2 diabetes, glucose oxidation is inappropriately low, possibly the result of the effect of elevated non-esterified ('free') fatty acids on PDH. Fatty acid oxidation results in elevated acetyl-CoA/CoA, ATP/ADP and NADH/NAD⁺ ratios, which increase PDHK activity. In addition, fatty acids exert a more long-term effect on glucose oxidation by increasing the expression of the high-specific-activity isoform PDHK4, which may occur at least in part via activation of peroxisome-proliferator-activated receptor α [1]. Diabetes is also associated with elevated hepatic glucose production, so activation of PDH may have the potential to decrease blood glucose, not only by increasing glucose oxidation, but also reducing the supply of the gluconeogenic substrates lactate and alanine.

DCA (dichloroacetate) is a non-specific inhibitor of PDHK which has been shown to increase both muscle and liver PDH activity in a number of rat models of diabetes: streptozotocin- [2] and alloxan-induced [3] insulin-deficient states and models of type 2 diabetes such as the dexamethasone-induced [4] and the ZDF (Zucker diabetic fatty) rat [5]. In these models, with the exception of alloxan-diabetes, DCA causes a decrease in blood glucose. Clinically, DCA has shown some effect in hyperglycaemic patients [6]; consistent with the hypothesis that PDH activation will decrease the availability of gluconeogenic substrates, glucose lowering was associated with a marked decrease in plasma lactate and alanine. In the normal fed rat or non-diabetic human, PDH activation by DCA does not affect glucose levels, but it does lower glucose in fasted animals [7]. DCA is unsuitable as a therapeutic agent because of low potency, metabolism and toxicity [8].

The search for novel, small-molecule inhibitors of PDHK offering improved potency and specificity has been ongoing for some years. Halogenated acetophenones were described in 1995 [9]; these were of relatively low potency (>1 µM) and do not seem to have been developed further. Several other structural types have been identified by high-throughput screening techniques; these and their proposed mechanisms of action are reviewed in [10].

Activation of PDH in vitro
Compound screening at AstraZeneca has identified a novel series of anilide tertiary carbinols, including AZD7545, which are potent and specific inhibitors of PDHK2. AstraZeneca utilized a functional enzyme assay where native porcine PDH...
Effect of AZD7545 on PDH activity

The obese \((fa/fa)\) Zucker rat is a frequently used model of the insulin-resistant or prediabetic state. It exhibits impaired glucose tolerance, hyperphagia, hyperinsulinaemia and hyperlipidaemia. While not overtly hyperglycaemic, the \(fa/fa\) rat exhibits an abnormal glucose profile following feeding compared with its lean counterpart (4 h into the dark feeding phase, blood glucose levels are 8.7 mM, compared with 6.1 mM in lean animals). This is associated with a small but consistent and significantly elevated glycated haemoglobin level (3.49 versus 3.26%). At the age used in our study (12 weeks), PDH activity in the \(fa/fa\) rat was elevated compared with that in lean Zucker or Wistar rats. We have measured no difference in expression levels of PDHK2 or PDHK4 between obese and lean Zucker rats [15]. As in Wistar rats, PDH in fed \(fa/fa\) rats can be further activated by PDHK inhibitors (for example 10 mg/kg AZD7545 increases muscle PDH from 61.0 to 97.0% active, and liver PDH from 33.5 to 72.8%).

Obese Zucker rats were treated with the PDHK inhibitor AZD7545 orally for 7 days, and at the end of this period the glucose profile was monitored for 24 h (Figure 2). In control, vehicle-treated rats, blood glucose rose to a maximum of 9.45 ± 1.11 mM, whereas in rats treated with AZD7545 once daily at 08:00 h, the concentration was 6.55 ± 0.58 mM at the

**Effect of AZD7545 in Zucker \((fa/fa)\) rats**

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Figure 2 | Effect of AZD7545 on 24 h glucose profile

Obese male (fa/fa) Zucker rats, housed in a 06:00 h on/18:00 h off light cycle, were dosed for 7 days with either 10 mg/kg AZD7545, given orally, at 08:00 h (C) or 08:00 and 18:00 h (A) or with vehicle (■). On day 8, glucose was measured using a hand-held glucose monitor (Glucotrend). The same time. A similar obliteration of the postprandial glucose elevation was seen after administration twice daily.

This is the first report of the testing of a novel PDHK inhibitor in the obese Zucker rat and provides clear evidence that a PDHK inhibitor can improve the control of blood glucose levels in an animal model with impaired glucose homeostasis. This is in contrast to the statement by Aicher et al. [14] that small-molecule inhibitors were ineffective in animal models of diabetes (ob/ob mice and ZDF rats). Clearly our data would suggest that an inhibitor of PDHK will be an effective novel therapy for type 2 diabetes.

References


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