PDH kinase inhibitors: a novel therapy for Type II diabetes?

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Abstract
The pyruvate dehydrogenase multienzyme complex catalyses the oxidative decarboxylation of pyruvate, which is an important regulatory step in oxidative metabolism. Phosphorylation of the E1 (pyruvate decarboxylase) subunit on one of three specific serine residues results in loss of enzyme activity. Four dedicated PDHK (pyruvate dehydrogenase kinase) isoenzymes have been identified, each of which display a distinct tissue-specific expression profile, and have differential regulatory properties. Thus PDHK play a key role in controlling the balance between glucose and lipid oxidation according to substrate supply. Increasing glucose oxidation by inhibiting PDHK may be an effective mechanism to increase glucose utilization; additionally, increasing pyruvate oxidation may further contribute to lowering of glucose level by decreasing the supply of gluconeogenic substrates. A number of PDHK inhibitors are now available to enable this mechanism to be evaluated as a therapy for diabetes. The isoenzyme selectivity profile of AZD7545 and related compounds will be described and evidence for their non-ATP-competitive mode of action presented. These compounds increase PDH activity in vivo, and when dosed chronically, improve glycaemic control in Zucker rats. Furthermore, glucose lowering has been demonstrated in the hyperglycaemic Zucker diabetic fatty rat. This result supports the hypothesis that inhibition of PDHK may be an effective therapy for Type II diabetes.

Introduction
PDH (pyruvate dehydrogenase) catalyses the irreversible oxidation of pyruvate, yielding acetyl-CoA and CO₂, and is a key enzyme in controlling the rate of oxidative glycolysis and regulating the balance between oxidation of carbohydrate and lipid fuels. The activity of PDH is tightly controlled by a balance between the activities of specific PDHK (PDH kinase) and PDH phosphatase enzymes. Activation of PDH should benefit the diabetic state by inhibiting gluconeogenesis and promoting glucose disposal in peripheral tissues. Preliminary evidence in support of this proposal was obtained using DCA (dichloroacetate) but this compound is unsuitable as a therapeutic agent for diabetes because of low potency, metabolism and toxicity [1].

The search for novel, small-molecule inhibitors of PDHK offering improved potency and selectivity has been ongoing for some years, and recently compounds have been identified that activate PDH in vitro, which have been used as tools to investigate further the potential of this mechanism as a therapy for Type II diabetes. Aicher et al. [2] reported a series of compounds which activate PDH in vitro and decrease lactate in vivo; one of these, identified as ‘Novartis 3r’, has proved to be of particular interest in evaluating in vivo efficacy. We described AZD7545 and related compounds [3,4], which have structural similarities to those described by Aicher et al. [2]. Further exploration of the in vitro selectivity and in vivo properties of these compounds is discussed in this review.

PDH in diabetes
Type II diabetes is associated with alterations in the balance between glucose and lipid metabolic pathways [5]. Excessive hepatic glucose production, largely from gluconeogenesis, accounts for a significant proportion of hyperglycaemia in Type II diabetics with fasting blood glucose levels of >10 mM [6]. Decreasing the supply of gluconeogenic substrates, by increasing the oxidation of pyruvate in peripheral tissues, is an attractive mechanism for lowering this excessive gluconeogenic rate. DCA, a prototype PDHK inhibitor, decreases alanine flux and lactate levels in humans by increasing pyruvate oxidation rate in peripheral tissues. However, in normal subjects, there is no increase in hepatic lactate clearance [7]. In contrast, in a limited study in Type II diabetic patients, DCA decreased glucose concentration in association with a reduction in lactate and alanine and this was, not surprisingly, most marked in those patients with high glucose levels [8].

Activation of PDH in skeletal muscle will increase the rate of glucose oxidation and hence glucose disposal. Basal glucose oxidation rate is not impaired in Type II diabetes (for example [9]) but may be inappropriately low for elevated levels of glucose, and furthermore diabetic patients have an impaired ability to increase glucose oxidation in response to hyperinsulinemia [10]. The role of PDH in the pancreatic β-cell is less clear. Augmenting glucose oxidation and hence ATP production may be important in promoting glucose-stimulated insulin secretion from the pancreas through the
activation of ATP-sensitive K⁺ channels [11], although when PDHK and PDH phosphatase were overexpressed in MIN6 cells, glucose-stimulated insulin secretion was not affected [12,13], which apparently contradicts this hypothesis. Furthermore, exposure of rat islets to fatty acids increased glucose-stimulated insulin release despite decreased PDH activity [12].

**Regulation of PDH by specific kinase isoforms**
The PDH multienzyme complex consists of multiple copies of three catalytic subunits, E1 (pyruvate decarboxylase), E2 (dihydrolipoamide acetyltransferase) and E3 (dihydrolipoamide dehydrogenase) in conjunction with the E3 binding protein. Three specific serine residues on the E1 subunit may be phosphorylated, any one of which results in complete inhibition of enzyme activity [14]. Four distinct PDHK isoenzymes have been isolated, which share a high degree of sequence identity (66–74%); however, they each display distinct regulatory properties and tissue distributions [14,15]. In rats, PDHK2 is ubiquitously expressed, PDHK1 is restricted to the heart and PDHK3 is largely restricted to the testis, although with low abundance [16]. PDHK4 is of particular interest for diabetes as it is expressed at high levels in heart and skeletal muscle, and to a lesser extent in liver and is rapidly up-regulated in response to starvation. The tissue distribution of the PDHK isoforms in human tissues largely reflects that found in rats, although there is some trace of PDHK1 in skeletal muscle; PDHK3 appears in heart and skeletal muscle and PDHK4 appears to be almost nonexistent in liver [17,18]. Isoform-specific differences in regulation, distribution and specific activity have led to the concept that there are marked tissue-specific differences in the regulation of PDH activity, which are critical for maintaining whole-body homeostasis [19].

**PDHK isoform expression in diabetes**
Although PDHK4 is markedly up-regulated in skeletal muscle in animal models of Type I diabetes [20], the situation in insulin resistance and Type II diabetes is less clear. In obese Zucker rats, expression levels of PDHK2 and PDHK4 in liver and skeletal muscle are similar to those in lean littermates [21]; however, high-fat feeding of Wistar rats [22] increased kinase expression, particularly of PDHK4 in soleus muscle. Similarly, the mildly hyperglycaemic but highly insulin-resistant Otsuka Long–Evans Tokushima Fatty rat showed increased levels of both mRNA and protein for PDHK2 and PDHK4 when compared with younger, less insulin-resistant animals and lean counterparts [23]. These apparent discrepancies may arise from the use of different muscle groups, as fibre-type-specific differences in PDHK expression have been reported [24,25]. Studies to investigate the level of muscle PDH activity in Type II diabetes or obesity suggest that this is either unaltered or slightly elevated compared with normal subjects, but results are variable, perhaps due to methodological difficulties. In a recent study, a group of hyperglycaemic patients (11.4 mM fasting glucose) with unimpaired basal glucose oxidation were shown to have normal expression of PDHK4 [9]. In contrast, a high fat, low carbohydrate diet for 3 days significantly increased PDHK activity and PDHK4 expression, with a corresponding decrease in PDH activity [26].

Expression levels of PDHK isoforms in liver in diabetic and insulin-resistant states are not well documented. No difference has been observed between lean and obese Zucker rats [21]. Perhaps not surprisingly, there are no reports of liver PDH activity or PDHK isoform expression in diabetic patients.

**Isoform selectivity in the treatment of diabetes**
PDHK4 may be an attractive therapeutic target because this isoform is specifically expressed in skeletal muscle and heart, which are major sites of glucose disposal. Activation of PDH in these tissues would increase glucose utilization and decrease the supply of gluconeogenic intermediates from peripheral tissues; furthermore, it would avoid the possibility that increased acetyl-CoA production in the liver would stimulate lipogenesis. However, this approach bypasses the potential benefit of increasing PDH activity in the liver itself. Furthermore, prevention of the ‘metabolic switch’ [10] that enables essential tissues such as the heart to rely on fatty acids as a fuel source in situations of substrate depletion could be deleterious. Indeed, mice with a deletion of the PDHK4 gene develop hypoglycaemia on fasting [27]. An alternative profile is inhibition of PDHK2 in the absence of PDHK4. This would increase glucose uptake and decrease gluconeogenesis in the postprandial state when PDHK2 is the predominant isoenzyme in peripheral tissues as well as liver. PDHK4 is rapidly up-regulated following a few hours of fasting [28] and will thus maintain protection against excessive utilization of carbohydrate substrates. We have no evidence that excessive hepatic lipogenesis occurs in the presence of PDHK2 inhibition.

**AZD7545 and related PDHK inhibitors: selectivity for PDHK isoforms**
We have developed AZD7545 and a related series of PDHK2 inhibitors. These compounds activate PDH in a coupled enzyme assay and stimulate pyruvate oxidation in isolated rat hepatocytes; furthermore, they increase PDH activity in vivo and lower glucose levels in obese Zucker rats [3,4]. To explore the isoenzyme selectivity of these compounds, kinase assays for PDHK1, PDHK2 and PDHK4 were established. The IC₅₀ values for inhibition of PDHK2 and PDHK1 by AZD7545 were 6.4 ± 2.2 nM (n = 6) and 36.8 ± 18 nM (n = 3) respectively. Other compounds in this series, and Novartis 3r, inhibited both PDHK1 and PDHK2, although a consistent trend of decreased potency (5–15-fold) towards PDHK1, as compared with PDHK2, was observed (Table 1). In contrast,
Inhibition of PDHK isoenzymes by AZD7545 and related compounds

The incorporation of [33P]ATP into the E1 subunit of PDH was measured at 30°C in Mops-K+ buffer [33] in the presence of 100 µM ATP and E2 subunit. Recombinant human proteins were used throughout. IC50 values are geometric means ± S.E.M. with the number of observations in parentheses. incr., increased.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>PDHK1</th>
<th>PDHK2</th>
<th>PDHK4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD7545</td>
<td>36.8 ± 18 (3)</td>
<td>6.4 ± 2.2 (6)</td>
<td>incr.</td>
<td></td>
</tr>
<tr>
<td>Compound K</td>
<td>15.9 ± 9.3 (5)</td>
<td>1.4 ± 0.4 (4)</td>
<td>incr.</td>
<td></td>
</tr>
<tr>
<td>Novartis 3r [32]</td>
<td>32.0 ± 8.2 (3)</td>
<td>4.8 ± 0.3 (4)</td>
<td>incr.</td>
<td></td>
</tr>
<tr>
<td>DCA*</td>
<td>1000</td>
<td>200</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

*Ki (app) for DCA (µM) from [16].

these novel PDHK inhibitors failed to inhibit PDHK4 and paradoxically stimulated this isoenzyme (results not shown). In contrast, DCA inhibits all PDHK isoforms [14,16].

**Isoform selectivity in vivo**

An interesting observation is that administration of maximally effective doses of AZD7545 and related compounds to Wistar rats results in the near-complete activation of PDH activity in liver, where PDHK2 is the major isoenzyme, whereas only partial activation of PDH activity is achieved in skeletal muscle and heart, tissues which express high levels of PDHK4. For example, after the administration of a maximally effective dose of compound K (a more potent inhibitor than AZD7545), the percentage of PDHα in the active (dephosphorylated) state in liver is elevated from 35.3 ± 4.0 to 90.2 ± 2.2%, whereas in skeletal muscle and heart, PDH activity plateaus at 64.3 ± 2.3 and 61.8 ± 4.3% respectively. Further evidence for a link between in vitro isoenzyme selectivity and in vivo activity comes from the observation that in fasted rats the ability of AZD7545 to elevate PDH activity in liver is intact, whereas the activation of PDH in skeletal muscle in response to compound is severely blunted [3]. A similar result has been obtained with Novartis 3r (R.M. Mayers, unpublished work), whereas DCA activates muscle PDH to equivalent levels in both fasted and fed rats [28].

**Glucose lowering in animal models of diabetes**

The obese (fa/fa) Zucker rat is frequently used as a model of the insulin-resistant, or prediabetic state. It exhibits impaired glucose tolerance, hyperphagia, hyperinsulinaemia and hyperlipidaemia. Although not overtly hyperglycaemic, the fa/fa rat exhibits an abnormal glucose profile after feeding when compared with its lean counterpart, which is associated with a small but consistent and significantly elevated glycated haemoglobin level. Treatment with the PDHK inhibitor AZD7545 results in a near-complete ablation of this post-prandial glucose elevation [4]. Furthermore, AZD7545, administered for 4 weeks, had no effect on food intake or body weight gain, in contrast with the effect of the standard novel antidiabetic agent rosiglitazone, in the same study [29]. Plasma triacylglycerol and circulating fatty acid levels were unchanged; however, on fasting, fatty acid levels were slightly elevated compared with control animals (results not shown).

A number of PDHK inhibitors have been tested in hyperglycaemic animal models. DCA, dosed to 12-week-old ZDF rats increased the PDH activity in tibialis anterior and cardiac muscle, kidney and liver, and decreased glucose levels, with sustained efficacy after 25 days of dosing [30]. However, the same authors tested two novel PDHK inhibitors [30–32] in 24 h fasted, 8-week-old ZDF rats. Significant increases in PDH activity were demonstrated in the same four tissues, but these were, however, of smaller magnitude than reported for DCA (Table 2), and although plasma lactate levels were decreased, no glucose lowering was observed over an 11 day dosing period. This is consistent with the relatively low increase in PDHα, which might be a consequence of potency and/or pharmacokinetic properties of the compound, or, alternatively, its isoenzyme selectivity. In this study, the percentage of PDHα in the active form was not determined, but is likely to be low for muscle tissues due to elevated PDHK4 expression induced by fasting.

In contrast, we have demonstrated sustained efficacy in overtly hyperglycaemic ZDF rats. Compound K was administered for 14 days to 12-week-old ZDF rats, which had prefeeding glucose levels of 21.7 ± 2.1 mM. A single, oral dose of compound K reduced the glucose excursion in response to the start of feeding on day 1 from 4.2 ± 1.1 to 2.4 ± 0.7 mM (P = 0.09) and this improvement was sustained over 14 days of dosing (Figure 1). It is possible that the apparent contradiction between our own results and those of Aicher et al. [32] may arise from methodological differences.

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**Table 1 | Inhibition of PDHK isoenzymes by AZD7545 and related compounds**

<table>
<thead>
<tr>
<th>Compound</th>
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<td>DCA*</td>
<td>1000</td>
<td>200</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

*Ki (app) for DCA (µM) from [16].

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**Table 2 | Activation of PDH in tissues of male ZDF rats**

For 3r and 10a, PDH activity was measured ex vivo in tissues from 24 h fasted ZDF rats, using a coupled enzyme assay, and normalized for citrate synthase activity.

<table>
<thead>
<tr>
<th>Fold increase in PDHα</th>
<th>DCA [30]</th>
<th>3r [32]</th>
<th>10a [31]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis anterior</td>
<td>9</td>
<td>5.5°</td>
<td>2.8°</td>
</tr>
<tr>
<td>Heart</td>
<td>30</td>
<td>2.5°</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>30</td>
<td>1.4°</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>8</td>
<td>2.4°</td>
<td>2.8°</td>
</tr>
</tbody>
</table>

*The increase in activity is an approximate value derived from a figure in the indicated publications. For DCA, methodological details are assumed to be similar.

†Value differs (P < 0.05) from the corresponding value for control animals.
Figure 1 Effect of compound K on glucose concentration in male ZDF rats

Obese male ZDF rats, 12 weeks old at the start of the study, were dosed orally once daily 2 h prior to the dark phase with vehicle (■), rosiglitazone (▲) or compound K (●). Blood glucose levels were measured daily, immediately before dosing, from a tail prick sample using a hand-held glucose monitor.

These authors do not give full experimental details; but, for example, increase in PDHK4 induced by an overnight fast may mean that PDH activity cannot be increased sufficiently to influence glucose levels.

Evidence is therefore accumulating that specific and selective inhibitors of PDHK are effective in improving glycaemic control in a number of animal models of diabetes and would suggest that activation of PDH may be a valuable novel therapeutic mechanism for the treatment of Type II diabetes. Further investigation is required to develop our understanding of the impact of influencing metabolic flexibility in insulin resistance and diabetes. Identification of compounds with known selectivity has also provided useful tools for the evaluation of the role of specific kinase isoforms in specific tissues and disease states.

References


Received 1 November 2004