AZD7545 is a selective inhibitor of pyruvate dehydrogenase kinase 2

J.A. Morrell*, J. Orme*, R.J. Butlin*, T.E. Roche†, R.M. Mayers* and E. Kilgour*1

* AstraZeneca, Alderley Park, Macclesfield, Cheshire, SK10 4TG, U.K. and †Department of Biochemistry, Kansas State University, Manhattan, KS, U.S.A.

Abstract

The PDH (pyruvate dehydrogenase) multi-enzyme complex catalyses a key regulatory step in oxidative glycolysis. Phosphorylation of the E1 subunit of the complex on serine residues results in the inactivation of enzyme activity. A family of four dedicated PDH kinase isoenzymes exists, each of which displays a distinct tissue-specific expression profile. AZD7545 is one of a series of PDH kinase inhibitors developed for the treatment of type 2 diabetes. The isoenzyme-selectivity profile of AZD7545 and related compounds is described and the consequences for their in vivo mode of action are discussed.

Introduction

The mitochondrial PDH (pyruvate dehydrogenase) complex catalyses the irreversible decarboxylation of pyruvate to acetyl-CoA, a key regulatory step in the control of glucose utilization. This has led to the hypothesis that a PDH activator will increase glucose disposal and be an effective glucose-lowering approach for treatment of type 2 diabetes. PDH is inactivated by reversible phosphorylation and the enzyme activity in tissues is regulated by two classes of dedicated enzyme, the PDHKs (PDH kinases) and the PDH phosphatases.

The development of PDHK inhibitors represents a practical approach to PDH activation and this review describes the characterization of the isoenzyme selectivity of AZD7545 and related PDHK inhibitors and explores the link with the in vivo activity of these compounds.

PDHK isoenzymes

The PDH multi-enzyme complex consists of multiple copies of three catalytic subunits, E1 (pyruvate decarboxylase), E2 (dihydrolipoamide acetyltransferase) and E3 (dihydrolipoamide dehydrogenase) along with the E3-binding protein. Three specific serine residues on the E1 subunit are phosphorylated by PDHKs resulting in complete inhibition of enzyme activity [1]. Four distinct PDHK isoenzymes have been isolated and shown to lack the signature sequence motifs found in other serine/threonine kinases, but instead share some similarity with the bacterial histidine kinases. Although a high degree of sequence identity (66–74%) exists between the four PDHK isoenzymes they each display distinct regulatory properties and tissue distributions [1,2]. Whereas PDHK2 is expressed ubiquitously, PDHK1 is largely restricted to the heart and PDHK3 is highly enriched in the testis. PDHK4 is of particular interest for diabetes as it is expressed at high levels in heart and skeletal muscle, the latter of which represents a major site for glucose disposal. Furthermore, expression of this isoenzyme is regulated in response to metabolic status [1,2]; PDHK4 expression is up-regulated in starvation, in response to high-fat feeding, in streptozotocin-treated rats and in skeletal muscle of the Zucker diabetic fatty rat and is down-regulated by insulin and peroxisome proliferator-activated receptor γ agonists [1–3]. Thus pharmacological modulation of the PDHK isoenzymes provides some potential for activation of PDH in a tissue-specific manner.

AZD7545 and related PDHK inhibitors: in vitro mode of action

We have developed AZD7545 and a related series of PDHK2 inhibitors. These compounds stimulate pyruvate oxidation in isolated rat hepatocytes, increase PDH activity in vivo and lower glucose levels in obese Zucker rats (see Mayers et al. [4]). In order 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 (Table 1). Other compounds in this series inhibited both PDHK1 and PDHK2 and a consistent trend of reduced potency (5–15-fold) towards PDHK1, as compared with PDHK2, was observed (Table 1). In contrast, AZD7545 and related compounds failed to inhibit PDHK4 and paradoxically, at higher concentrations (>10 nM), AZD7545 stimulated PDHK4 activity (results not shown).

To further understand the mode of action of the compounds the effect of ATP concentration on inhibition of PDHK2 was investigated. The IC50 for inhibition of PDHK2 by AZD7545 was unaffected when the concentration of ATP in the assay was varied between 10 µM (the Km value for
Table 1 | Inhibition of PDHK isoenzymes by AZD7545 and related compounds

The incorporation of \( ^{33}\text{P}\)ATP into the E1 subunit of PDH was measured at 30°C in Mops-K\(^+\) buffer [8] in the presence of 100 \( \mu \text{M}\) ATP and E2 subunit. Recombinant human proteins were used throughout. Compounds J, K and L are from the same chemical series as AZD7545. IC\(_{50}\) values are means ± S.E.M. with the number of observations given in parentheses.

<table>
<thead>
<tr>
<th>Compound</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>↑</td>
</tr>
<tr>
<td>Compound J</td>
<td>10.5 ± 2.4 (4)</td>
<td>0.6 ± 0.1 (5)</td>
<td>↑</td>
</tr>
<tr>
<td>Compound K</td>
<td>15.9 ± 9.3 (5)</td>
<td>1.4 ± 0.4 (4)</td>
<td>↑</td>
</tr>
<tr>
<td>Compound L</td>
<td>39.9 ± 13.2 (5)</td>
<td>2.6 ± 0.7 (4)</td>
<td>↑</td>
</tr>
</tbody>
</table>

PDHK2; IC\(_{50}\) = 6.1 ± 1.2 nM, \( n = 5 \) and 100 \( \mu \text{M}\) (10-fold above \( K_m \); IC\(_{50}\) = 6.4 ± 2.2 nM, \( n = 6 \)). Similar data were obtained for other compounds in this series (results not shown) and therefore it can be concluded that they inhibit PDHK in a non-ATP competitive manner. If the compounds are not interacting at the ATP-binding site what then is their site of action? Phosphorylation of E1 by PDHK is dependent upon E2. PDHK binds to the lipoic domains of E2 which results in activation of the kinase and facilitates encounters between the kinase and E1 [1,4]. One possibility, currently under further investigation, is that AZD7545 docks into the lipoamide-binding site on PDHK2 thus inhibiting interaction with the E2 subunit.

AZD7545 and related PDHK inhibitors: in vivo activity

One obvious question is how isoenzyme selectivity of these compounds relates to their activity in vivo. An interesting observation is that administration of maximally effective doses of AZD7545 and related compounds to rats results in the near-complete activation of PDH activity in liver, where PDHK2 is the major isoenzyme, while only partial activation of PDH activity is achieved in skeletal muscle and heart, tissues which express high levels of PDHK4 [6,7]. For example, following administration of a maximally effective dose of compound K (30 mg/kg) the percentage of PDH present in the active (dephosphorylated) state in liver is elevated from 35.3 ± 4.0% to 90.2 ± 2.2% while 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, while the activation of skeletal muscle PDH activity in response to the compound is severely blunted (Figure 1). During starvation the expression of PDHK4 is known to be greatly elevated in skeletal muscle but not liver [1,2] and this may explain the loss of efficacy in the former.

Conclusions

AZD7545 and related compounds effectively inhibit PDHK2 and PDHK1 but not PDHK4 activity. This isoenzyme selectivity correlates with the ability of these compounds in vivo to elevate PDH activity more effectively in liver than...
in skeletal muscle and heart and with the loss of efficacy in skeletal muscle of fasted animals. Further evaluation of these compounds in a range of metabolic/hormonal states should help to further our understanding of the role of PDHK isoenzymes in the control of whole-body metabolic homoeostasis.

References


Received 4 July 2003