Chiral inversion of 2-arylpiononyl-CoA esters by human α-methylacyl-CoA racemase 1A (P504S)—a potential mechanism for the anti-cancer effects of ibuprofen

Timothy J. Woodman, Pauline J. Wood, Andrew S. Thompson, Thomas J. Hutchings, Georgina R. Steel, Ping Jiao, Michael D. Threadgill and Matthew D. Lloyd*

Received 9th February 2011, Accepted 26th April 2011
DOI: 10.1039/c1cc10763a

Metabolic chiral inversion of 2-arylpionic acids (2-APAs; ‘profens’), such as ibuprofen, is important for pharmacological activity. Several 2-APA-CoA esters were good racemisation substrates for human AMACR 1A, suggesting a common chiral inversion pathway for all 2-APAs and an additional mechanism for their anti-cancer properties.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in human and veterinary medicine for their analgesic and anti-inflammatory properties. They inhibit cyclo-oxygenase-1 and -2 (COX-1 and -2), reducing production of prostaglandins and other inflammatory mediators. COX-1 is present in all tissues, while COX-2 levels are normally low but increase in response to tissue injury. Common examples include aspirin, indomethacin, diclofenac and the 2-arylpionic acids (2-APAs; a.k.a. profens), including ibuprofen. 2-APA drugs contain a chiral centre and their S-enantiomers potent inhibit COX activity, while the R-enantiomers are much less active. These drugs are often given as racemic mixtures. Ibuprofen, fenoprofen, flubufen, flurbiprofen, ketoprofen and naproxen undergo uni-directional conversion to the single S-enantiomer in vivo. R-Ibuprofen 1 is converted to R-ibuprofenoyl-CoA 2 undergo uni-directional conversion to the single S-enantiomer in vivo. R-Ibuprofen 1 is converted to R-ibuprofenoyl-CoA 2 and chirally inverted by an epimerase. Finally, hydrolysis of the CoA ester gives S-ibuprofen 1. Other 2-APAs are assumed to be inverted by the same enzymes as for ibuprofen, but this has not been proven.

Cloning of ‘ibuprofenoyl-CoA epimerase’ showed that it was identical to α-methylacyl-CoA racemase (AMACR), which also catalyses chiral inversion of other 2-methyl-fatty acyl-CoA esters. The reaction involves removal of the substrate α-proton followed by non-stereoselective repromotion. Protein levels and activity are increased in prostate, some colon and other cancers, and AMACR has attracted interest as a novel marker and drug target.

Although the acyl groups of AMACR substrates are known to be structurally diverse, no systematic study of substrate structure has been reported. This communication reports the first such systematic study and shows that all of the tested 2-APA-CoA esters are good substrates. High doses of ibuprofen reduce the risk of developing prostate cancer and these results suggest that the interaction of 2-APA-CoA esters with AMACR may contribute to this chemopreventive effect.

Initially, a number of 2-APA-CoA esters were synthesized for evaluation. Ibuprofenoyl-CoA 2 was chosen, as a known substrate for native rat AMACR and the homologous MCR from M. tuberculosis. 21 ±-Flurpropofenoyl-CoA 7, ±-flurbiprofenoyl-CoA 8, and S-ketopofenoyl-CoA 9 were synthesized, as these 2-APAs undergo chiral inversion in vivo. S-Naproxenoyl-CoA 10 was also synthesized, as it is structurally related but its chiral inversion in vivo is reported to be extremely limited. 11 The 2-APAs reacted with carbonyl diimidazole to form the acyl-imidazole intermediates, the presence of which was confirmed by 1H NMR. These intermediates were immediately treated with CoA-SH, to afford the desired esters on a 10 mg scales (ESI, Scheme S1). S-2-Methyldecanoyl-CoA 11 was also synthesized as a known substrate of human recombinant AMACR 1A.

These acyl-CoA esters were incubated at 400 μM with human AMACR 1A in the presence of 2H2O for >16 h and activity was monitored by exchange of the α-proton for 2H by 1H NMR. Exchange of the α-proton for 2H at ca. 70-90% conversion was observed for all 2-APA-CoAs. The signal for the methyl group on C-2 appears as an asymmetrical doublet (Fig. 1), composed of the unresolved triplet of the 2H-labelled product superimposed on the doublet of unreacted substrate. There was also a reduction in the intensity of the signal for 2H. The known substrate, S-2-methyldecanoyl-CoA 11, was converted >95% under the same conditions. No exchange was observed in negative controls containing heat-inactivated enzyme.

Experiments were then performed to determine whether chiral inversion of the 2-APA-CoA had also occurred in addition to α-proton exchange. Thus, ca. 10 mg of S-ketopofenoyl-CoA 9 and S-naproxenoyl-CoA 10 were separately incubated with AMACR in 2H2O-containing buffer. The product CoA esters were hydrolysed and the 2-APAs were converted to the...
diastereomeric N-(S-1-phenylethyl)amides 12 and 13. The
$^1$H NMR spectra showed two overlapping series of peaks
corresponding to the 2-H, the 2-Me, the NCHMe and the amide
NH (Fig. 2B), showing that chiral inversion had occurred.
The ratio of the two epimeric products in both cases was close to 1 : 1,
consistent with observations for 2-methyldecanoyl-CoA 11
($K_{eq} = 1.09 \pm 0.14; S/R$). Similar treatment of the 2-APAs
without exposure to the enzyme gave single sets of signals,
confirming that the configuration of the chiral centre was
maintained during derivatization (Fig. 2A). Exchange of the
$\alpha$-proton is obligatory for chiral inversion and, since no exchange
is observed with heat-inactivated enzyme, this demonstrates that
chiral inversion is enzyme-catalysed.

Kinetic parameters for the 2-APA-CoA and S-2-methyl
decanoyl-CoA esters were then determined (Table 1). All the
2-APA-CoA esters were good substrates of human AMACR 1A.
Flurbiprofenoyl-CoA 8, ibuprofenoyl-CoA 2 and naproxenoyl-
CoA 10 were converted about as efficiently as S-2-methyl
decanoyl-CoA 11 (as judged by $k_{cat}/K_m$). The results also
suggest that the limited chiral inversion of flurbiprofen$^{10}$
in humans is due to low conversion to its CoA ester 8
in vivo rather than it not being a chiral inversion substrate.
Ketoprofenoyl-CoA 9 was converted ca. twice as efficiently
as S-2-methyldecanoyl-CoA 11. Fenoprofenoyl-CoA 7 was by
far the best substrate ($k_{cat}/K_m = ca. 1400 \text{ M}^{-1} \text{s}^{-1}$), largely
due to the very low estimated $K_m$ value (2.3 $\mu$M cf. 26–74 $\mu$M for
other substrates). Examination of the kinetic plots for 7 showed
near-saturation even at the lowest substrate concentrations and it
is possible that the $K_m$ value is significantly lower than estimated
by this assay. It was not possible to use significantly lower
substrate concentrations, as a tight binding situation would occur,
i.e. the concentrations of substrate and enzyme would be similar.
Interestingly, the $K_m$ for 7 is of similar magnitude to the $K_i$
values for the best inhibitors of the rat enzyme (ca. 0.9 $\mu$M)$^{20}$

Literature inhibitors of AMACR$^{20}$ increase the acidity of the
$\alpha$-proton using electron-withdrawing groups, to facilitate
deprotonation and mimic the deprotonated intermediate.
The aliphatic side-chain of S-2-methyldecanoyl-CoA 11 is
electron-donating and replacement with any aryl side-chain
will make the $\alpha$-proton more acidic. 2-APA-CoA substrates
are expected to be more tightly bound based on this argument
and lower $K_m$ values are observed. Substrates with aromatic
rings substituted with electron-withdrawing groups (e.g. 8) are
expected to have particularly acidic $\alpha$-protons and this may
explain its tight binding.

There is no reported structure for AMACR, but the
structure of the $M. tuberculosis$ homologue with a number of
acyl-CoA has been reported.$^{21}$ These structures show the CoA
moiety locked into the active site by a network of hydrogen
bonds and ionic interactions. The carboxylate thioester and
moiety locked into the active site by a network of hydrogen

![Fig. 1](#) 2H-Exchange of $\pm$-flurbiprofenoyl-CoA 8 by AMACR 1A,
showing change in methyl group splitting at $\delta$ 1.45 p.p.m. (A)
Heat-inactivated enzyme; (B) Active enzyme.

![Fig. 2](#) Chiral inversion of naproxenoyl-CoA 10 by AMACR 1A. The
spectra show the signals for the $\alpha$-proton of 1-phenylethylamine and
the NH of the amide linkage. (A) Without exposure to enzyme; (B)
Following exposure to AMACR 1A. The signals appear as two
superimposed doublets at 5.55 p.p.m. and two superimposed quintets
at 5.10 p.p.m.

**Table 1** Substrate kinetic parameters for AMACR 1A. The
parameters shown are derived using the Direct Linear Plot$^{24,25}$

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmol min$^{-1}$ mg$^{-1}$)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_m$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2$</td>
<td>74</td>
<td>9.36</td>
<td>0.0073</td>
<td>99</td>
</tr>
<tr>
<td>$7$</td>
<td>2.3</td>
<td>4.16</td>
<td>0.0033</td>
<td>1426</td>
</tr>
<tr>
<td>$8$</td>
<td>26</td>
<td>5.12</td>
<td>0.0040</td>
<td>156</td>
</tr>
<tr>
<td>$9$</td>
<td>52</td>
<td>19.2</td>
<td>0.0150</td>
<td>290</td>
</tr>
<tr>
<td>$10$</td>
<td>68</td>
<td>12.2</td>
<td>0.0095</td>
<td>140</td>
</tr>
<tr>
<td>$11$</td>
<td>277</td>
<td>39.3</td>
<td>0.0310</td>
<td>112</td>
</tr>
</tbody>
</table>

Post facto molecular modelling studies were undertaken to
try to rationalize the binding of 2-APA-CoAs to AMACR.
The structures of MCR complexed with S- or R-ibuprofenoyl-CoA 21 were used as starting models. Binding of the CoA moiety and chiral centre are identical for all models, regardless of their stereochemical configuration, e.g. R-ibuprofenoyl-CoA 7 (Fig. 3). In each case, the side-chain projects towards the methionine-rich surface. 21 In all but one case (M198/F194), the predicted side-chain binding residues in AMACR are identical to those in MCR (ESI† Fig. S1). Substrates with meta-substituted aromatic rings (e.g. 7 and 9) could have an additional interactions between them and the side of the active site funnel and this may account for their tighter binding or conversion with higher catalytic efficiency.

In summary, a panel of structurally diverse 2-APA-CoA esters were efficiently converted by AMACR. Chiral inversion probably occurs in both directions, with stereoselective formation of the CoA ester accounting for the specific R- to S- inversion in vitro. 2,6-11 AMACR is highly conserved across species and the pathway is probably common in mammals and other species. Chemoprotective effects of 2-APAs have been previously reported, but their effects were ascribed to binding to p75NTR or COX. 26-28 Reduction of AMACR activity is proposed as a treatment for prostate and other cancers and inhibition of activity may be an additional mechanism for the chemopreventive effects of 2-APAs. Use of the R-2-APA enantiomer to inhibit AMACR, rather than administering a racemic mixture, would be an example of chiral switching 29 to extend the usefulness of a known drug.

This work was supported by Cancer Research UK. Part of this work was performed for a Nuffield Summer Student Bursary (TJH), a Nuffield Science Bursary (GRS) and a Bath-Shandong Pharmacy & Pharmacology Scholarship (TJW). This work is dedicated to Mr David K. Lloyd (1936 to 2010). TJW, PJW, AST, MDT and MDL are members of the Cancer Research @ Bath network.

Notes and references

† Abbreviations used: AMACR 1A, α-methyllacy-CoA racemase, splice variant 1A; 2-APA, 2-arylpropionic acid; CoA, coenzyme A; COX, cyclooxygenase; MCR, α-methylacyl-CoA racemase (M. tuberculosis).


