Biochemical Pharmacology, Vol. 37, No. 19, pp. 3539–3542, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

RAPID COMMUNICATIONS

ENANTIOSPECIFIC FORMATION OF FENOPROFEN COENZYME A THIOESTER IN VITRO

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(Accepted 18 July 1988)

Many non-steroidal 2-arylpropionate anti-inflammatory drugs such as ibuprofen and fenoprofen exist as R and S enantiomers but are administered to man as racaemic compounds. Metabolic chiral inversion in vivo of the R to the pharmacologically active S enantiomer [1-2] is thought to proceed via formation of a co-enzyme A (CoA) thioester intermediate [3]. The consequences of CoA thioester formation maybe far reaching, for example the formation of xenobiotic acyl-CoA intermediates has led to alterations in lipid metabolism [4] which may contribute towards the mechanism by which hypolipidaemic drugs produce their effect. In addition, stereospecific incorporation of R fenoprofen into rat hepatocyte and adipocyte triacylgly-cerols has been recently reported [5]. These hybrid lipids may provide long lived tissue residues of the 2-arylpropionic acids. Although the enzyme responsible for the formation of xenobiotic CoA esters has not been identified the hepatic microsomal form of long chain acyl-CoA synthetase (E.C. 6.2.1.3) appears to function totally in the synthesis of complex lipids and has been indirectly implicated in the formation of 3-phenoxybenzoic acid-CoA [6] and ciprofibroyl-CoA [7]. In this study fenoprofen was used as a model compound to investigate the role of acyl-CoA synthetase in the formation of fenoprofen CoA thioesters.

MATERIALS & METHODS: The R and S enantiomers of fenoprofen obtained by resolution of racaemic fenoprofen were assayed for purity (R, 98.1% and S, 97.4%) as previously described [8]. [1-^{14}C] palmitic acid (50 mCi/mmol) and Matrex Gel Red A were purchased from Amersham (Australia) Pty. Ltd. and Amicon Corporation respectively. All other chemicals were obtained from Sigma Chemical Co. Acyl-CoA synthetase solubilized from rat liver microsomes (hooded Wistar) using Triton X-100 (20%, w/v) was bound to Matrex Gel Red A and poured into a glass column (1 x 10 cm) according to the technique of Merrill et al [9]. Protein content and enzymic activity of the bound protein were determined as previously described [10-11]. A reaction mixture (12.5 ml) containing 0.1 M Tris-HCl, 10 mM ATP, 30 mM $MgCl_2$, 0.1% Triton X-100, 5 mM dithiothreitol, 2 mM CoASH and either R fenoprofen (1 mM) or RS fenoprofen (2 mM) was continuously recycled through the column at room temperature (1 ml/min) for 4 hours. Aliquots (100 ul) of the column effluent were removed at various times and mixed with 0.5 ml of Doyles medium (isopropanol/heptane/sulphuric acid, 40:10:1). Ketoprofen (0.25 ug) was added as an internal standard and the samples extracted with 1 ml heptane and frozen in a dry ice/acetone bath. The organic phase was analyzed for fenoprofen using an enantiospecific HPLC technique [8]. The aqueous phase was stored for 1 week at -80°C following addition of dithiothreitol (final concentration 10 mM) and acidification of the the samples (0.4 ml, 12% $HClO_4$). The amount of fenoprofen - CoA in the aqueous phase was determined by alkaline hydrolysis of the CoA thioester and subsequent HPLC analysis of the released fenoprofen. On

thawing, ketoprofen (0.25 ug) was added and the samples alkalinized to pH 12 (1N,KOH) prior to heating at 55°C for 60 minutes. When cool, samples were adjusted to pH 2, extracted with Doyles medium and analyzed for fenoprofen enantiomers as previously stated.

RESULTS: The Matrex Gel Red A retained 10.4 - 12.5 mg protein/ml beads. The bound protein exhibited high acyl-CoA synthetase activity towards ^{14}C-palmitate and 87% of the substrate was converted to ^{14}C-palmitoyl CoA in 30 minutes. In the presence of CoASH the concentration of R fenoprofen in the medium declined by 78% over the 4 hour period. In contrast, no change was observed in the absence of CoASH (fig.1). Using conditions which promote alkaline hydrolysis of CoA thioester bonds 78% of the R fenoprofen removed during recirculation was recovered from the aqueous phase of the 4 hour sample. No free R fenoprofen was detected by HPLC in the aqueous phase prior to alkaline hydrolysis. In the presence of RS fenoprofen, R fenoprofen concentration selectively declined by 82% over the 4 hours however the concentration of S fenoprofen was unchanged (fig.2).

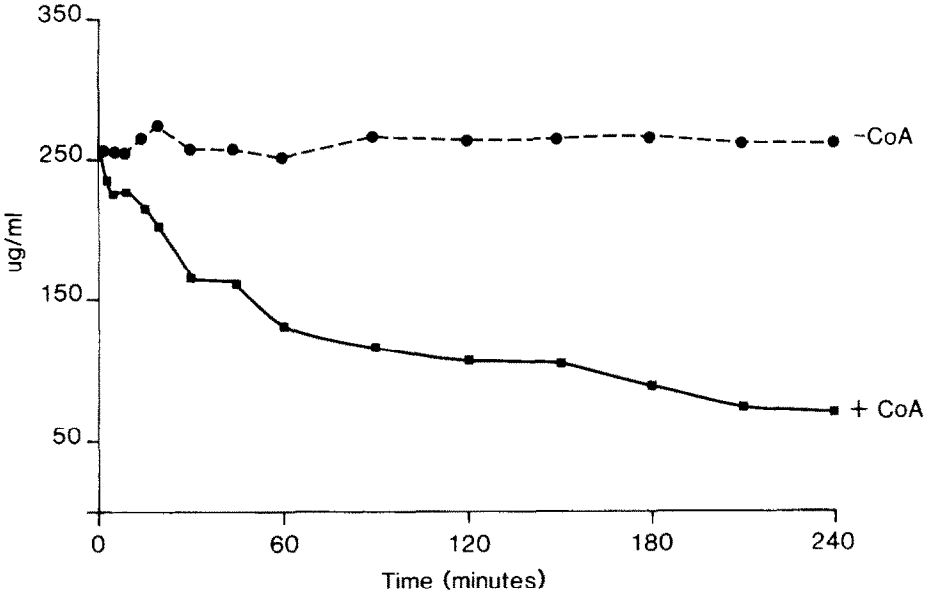

Figure 1. The effect of CoASH on the free concentration of R fenoprofen in the incubation medium.

DISCUSSION: The incorporation of fenoprofen into triacylglycerols is dependent on the initial activation of fenoprofen to the corresponding acyl-Coenzyme A and the subsequent stepwise esterification with glycerol. The synthesis of hybrid lipids has been reported to be stereospecific for the R enantiomers of fenoprofen [5] and ibuprofen [12], thus implying enantiospecificity of R-2arylpropionyl-CoA formation. Unlike previous studies which have identified lipids containing xenobiotics this is to our knowledge the first demonstration of fenoprofen-CoA thioester formation per se. In addition, the data provides evidence that activation of fenoprofen to the corresponding acyl coenzyme A is catalyzed by microsomal long chain acyl-CoA synthetase (E.C.6.2.1.3) and that the enzyme is stereospecific for the R enantiomer.

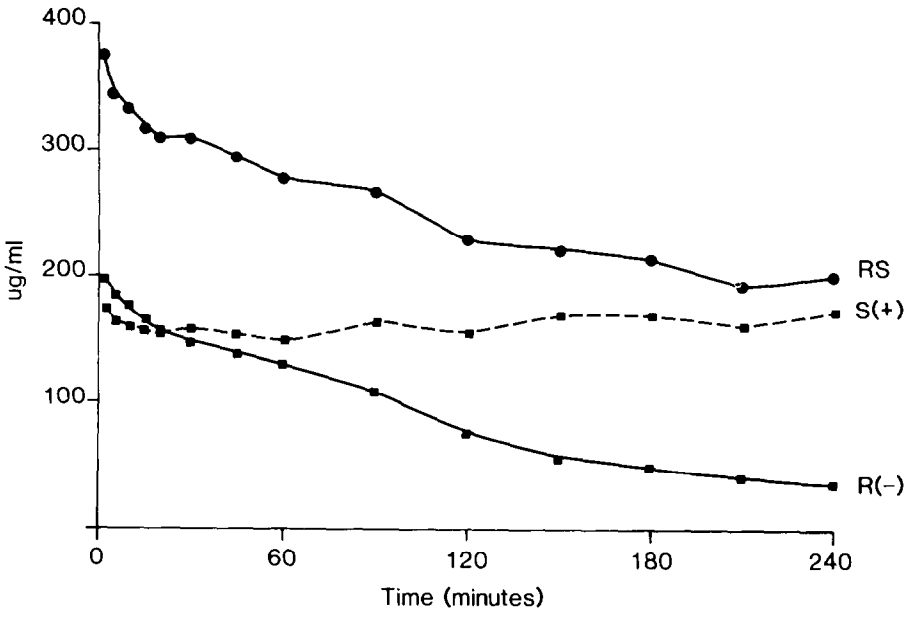

Figure 2. The selective decrease in the concentration of the R enantiomer in the presence of S fenoprofen.

ACKNOWLEDGEMENT: This work was supported by the Australian Arthritis Foundation.

REFERENCES:

1. Hayball PJ and Meffin PJ, Enantioselective disposition of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs. III Fenoprofen disposition. J. Pharmac. Exp. Ther. 240: 631-636, 1987.
2. Abas A and Meffin PJ, Enantioselective disposition of 2-arylpropionic acid non steroidal anti-inflammatory drugs. IV Ketoprofen disposition. J. Pharmac. Exp. Ther. 240: 637-641, 1987.
3. Nakamura Y, Yamaguchi T, Takahashi S, Hashimoto S, Iwatani K and Nakagawa Y, Optical isomerization mechanism of R(-)-hydratropic acid derivatives. J. Pharm. Dyn. 4: S-1, 1981.
4. Caldwell J, Marsh MV, Interrelationship between xenobiotic metabolism and lipid biosynthesis. Biochem. Pharmac. 32: 1667-1672, 1983.
5. Sallustio BC, Meffin PJ and Knights KM, The stereospecific incorporation of fenoprofen into rat hepatocyte and adipocyte triacylglycerols. Biochem. Pharmac. 37: In press 1988.
6. Imhoh DA, Logan CJ and Dodds PF, Synthesis of 3-phenoxybenzoic acid containing lipids via the monoacylglycerol pathway. Biochem. Pharmac. 34: 3009-3010, 1985.
7. Bronfman M, Amigo L and Morales HN, Activation of hypolipidaemic drugs to acyl-coenzyme A thioesters. Biochem. J. 239: 781-784, 1986.
8. Sallustio BC, Abas A, Hayball PJ, Purdie YJ and Meffin PJ, Enantiospecific high performance liquid chromatographic analysis of 2-phenylpropionic acid, ketoprofen and fenoprofen. J. Chromatog. 374: 329-337, 1986.
9. Merrill AH, Gidwitz S and Bell RM, Facile enzymatic synthesis of fatty acyl-coenzyme A thioesters. J. Lipid Res. 23: 1368-1373, 1982.

10. Bensadoun A, Weinstein D, Assay of proteins in the presence of interfering materials. Anal. Biochem. 70: 241-250, 1976.

11. Krisans SK, Mortensen RM and Lazarow PB, Acyl-CoA synthetase in rat liver peroxisomes. J. Biol. Chem. 255: 9599-9607, 1980.

12. Williams KM, Day RO, Knihinicki RD and Duffield A, The stereoselective uptake of ibuprofen enantiomers into adipose tissue. Biochem. Pharmac. 35: 3403-3405, 1986.