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Short Communication

Formation of 2,3-pristenic acid and 3-hydroxypristanic acid from pristanic acid in human liver

N. M. VERHOEVEN¹, D. S. M. SCHOR¹, G. A. JANSEN², R. M. KOK¹,
H. J. TEN BRINK¹, R. J. A. WANDERS² and C. JAKOBS^{1*}

¹*Department of Clinical Chemistry, Free University Hospital Amsterdam;* ²*Departments of Clinical Chemistry and Pediatrics, University of Amsterdam, Academic Medical Center, Amsterdam, The Netherlands*

**Correspondence: Department of Clinical Chemistry, Metabolic Unit, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands*

Studies concerning peroxisomal β -oxidation have mainly been performed using radio-labelled fatty acids, after which the end products, $^{14}\text{CO}_2$ and water-soluble radiolabelled products were measured (Wanders et al 1995). These studies only enable the investigation of a complete β -oxidation cycle and do not distinguish between the individual steps of β -oxidation. We have developed a method for investigation of the first two steps of peroxisomal β -oxidation of pristanic acid in human liver. After incubation of human liver homogenates with pristanic acid or pristanoyl-CoA, the intermediates formed were hydrolysed to give their free acids, derivatized, and quantified by stable-isotope dilution gas chromatography–mass fragmentography with negative chemical ionization. The applicability of the method described in this paper is demonstrated by showing the deficient oxidation of 2,3-pristanic acid to pristenic acid and 3-hydroxypristanic acid in liver from a Zellweger (McKusick 214100) patient.

MATERIALS AND METHODS

Preparation of human liver homogenates: Pieces of human liver from controls and Zellweger patients were stored at -70°C and thawed in a medium containing 250mmol/L mannitol, 5mmol/L Mops–NaOH and 1mmol/L EDTA (pH 7.4). Following homogenization in a glass homogenizer, the whole homogenates were used for enzyme studies.

Incubation conditions: The incubation mixture for studying pristanic acid metabolism contained, besides homogenized human liver (1mg protein): 150mmol/L KCl, 50mmol/L Hepes–NaOH, 2mmol/L K_3PO_4 and 50 $\mu\text{mol/L}$ of the substrate (pristanic acid or pristanoyl-CoA). To some incubations the following cofactors were added: 3mmol/L

Figure 1 Formation of pristanic acid and 3-hydroxypristanic acid from pristanic acid in liver homogenates from a control and a Zellweger patient, in the presence of ATP, Mg^{2+} and coenzyme A

$MgCl_2$, 2mmol/L ATP and 0.2mmol/L coenzyme A. The final pH was 7.4. Reactions were allowed to proceed for 60min at 37°C and were terminated by adding HCl (final concentration 0.33mol/L).

Pristanic acid, 2,3-pristanic acid, 3-hydroxypristanic, [2-methyl- 2H_3]pristanic acid, [2-methyl- 2H_3]pristanic acid and 3-hydroxy [2-methyl- 2H_3]pristanic acid were synthesized in the Free University Hospital Amsterdam. Pristanoyl-CoA was synthesized from pristanic acid using a previously described method (Rasmussen et al 1990).

Pristanic acid and 2,3-pristanic acid were measured as their pentafluorobenzyl derivatives and 3-hydroxypristanic acid was measured as its pentafluorobenzyl-acetyl derivative by stable-isotope dilution gas chromatography–mass fragmentography with negative chemical ionization. The derivatization procedure and analytical conditions were essentially as described before (Wanders et al 1994). Measurements were performed in the selected-ion monitoring (SIM) mode, measuring the fragments with m/z values 295 and 298 for pristanic acid and its internal standard and m/z 355 and 358 for 3-hydroxypristanic acid and its internal standard, respectively.

RESULTS AND DISCUSSION

When control human liver was incubated with pristanic acid, in the absence of ATP, Mg^{2+} and coenzyme A, no formation of 2,3-pristanic acid and 3-hydroxypristanic acid was observed. However, when ATP, Mg^{2+} and coenzyme A were added to the incubation mixture, formation of 2,3-pristanic acid amounted to about 170pmol/min per mg protein, whereas 3-hydroxypristanic acid was formed in lower amounts (2.4pmol/min per mg protein). Figure 1 shows that formation of pristanic acid and 3-hydroxypristanic acid is linear with time for at least 80min.

The necessity for coenzyme A in the incubation mixture implies that pristanic acid first

needs to be activated to pristanoyl-CoA before oxidation can occur. This was further investigated by using pristanoyl-CoA instead of pristanic acid as substrate. Higher rates of formation of intermediates were observed (210pmol/min per mg protein for pristanic acid and 4.5pmol/min per mg protein for 3-hydroxypristanic acid). As expected, when pristanoyl-CoA was used as substrate, the formation of intermediates was not influenced by added coenzyme A.

Figure 1 shows that formation of 2,3-pristanic acid and 3-hydroxypristanic acid was deficient in a liver homogenate from a Zellweger patient, irrespective of whether pristanic acid or pristanoyl-CoA was used as substrate.

The results described in this paper demonstrate the existence of the β -oxidation intermediates of pristanic acid: 2,3-pristanic acid and 3-hydroxypristanic acid. Furthermore, it is shown that activation of free pristanic acid to pristanoyl-CoA is essential for degradation of this compound.

In liver from a Zellweger patient, neither 2,3-pristanic acid nor 3-hydroxypristanic acid was found after incubation with pristanic acid or pristanoyl-CoA, which is in accordance with the elevated plasma levels of pristanic acid found in these patients (Ten Brink et al 1992) and the deficiency of pristanoyl-CoA oxidase which has been reported before (Wanders et al 1990).

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