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Review

Five decades with oxysterols

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ABSTRACT

I have been involved in research on oxysterols since 1963 and this review is intended to cover some of the most important aspects of this work.

The first project dealed with 7α-hydroxy-4-cholesten-3-one. My successful synthesis of this steroid with high specific radioactivity allowed a demonstration that it is a bile acid precursor. The mechanism of conversion of 7α-hydroxycholesterol into 7α-hydroxy-4-cholesten-3-one was investigated and I concluded that only one enzyme is required and that no isomerase is involved. Accumulation of 7α-hydroxy-4-cholesten-3-one in patients with lack of sterol 27-hydroxylase (*Cerebrotendinous xanthomatosis* was shown to be an important pathogenetic factor. This disease is characterized by cholestanol-containing xanthomas in tendons and brain and we could show that most of this cholestanol is formed from 7α-hydroxy-4-cholesten-3-one. We also showed that 7α-hydroxy-4-cholesten-3-one passes the blood–brain barrier.

In contrast to cholesterol itself, side-chain oxidized oxysterols have a high capacity to pass lipophilic membranes. We demonstrated conversion of cholesterol into 27-hydroxycholesterol to be a significant mechanism for elimination of cholesterol from macrophages. We also showed that conversion of cholesterol into 24S-hydroxycholesterol is important for elimination of cholesterol from the brain.

Side-chain oxidized oxysterols have a high capacity to affect critical genes in cholesterol turnover in vitro. Most of the published in vitro experiments with oxysteroids are highly unphysiological, however. Mouse models studied in my laboratory with high or low levels of 27-hydroxycholesterol have little or no disturbancies in cholesterol homeostasis. 24S-hydroxycholesterol is an efficient ligand to LXR and suggested to be important for cholesterol homeostasis in the brain. We recently developed a mouse model with markedly increased levels of this oxysterol in circulation and brain. This overexpression had however only a very modest effect on cholesterol turnover.

We concluded that oxysterols are not the master regulators of cholesterol homeostasis in vivo suggested previously.

1. Introduction

My first experience with oxysteroids started in 1963 when I was accepted as “research assistant” without salary at the department of Medical Chemistry at Karolinska Institutet. My tutor was associate professor Henry Danielsson, a previous pupil of Prof. Sune Bergström who later became a Nobel laureate.

This was before the era of molecular biology and the limitations in biochemical science were organic and analytical chemistry. The great scientific victories by Sune Bergström and his pupils were in particular based on organic chemistry: synthesis of new unlabelled and labelled steroids and fatty acids and their analyses by mass spectrometry. As a consequence of this the first project I became involved in was synthesis of a steroid that was believed but not proven to be an intermediate in the conversion of cholesterol into bile acids, namely 7α-hydroxy-4-cholesten-3-one.

This specific oxysteroid has followed me during my whole scientific career and I have published a number of studies concerned with different aspects on it: its chemical synthesis, the mechanism of its enzymatic formation, its metabolism, its role in bile acid biosynthesis, its role in pathogenetic mechanisms, its role as a marker for bile acid synthesis, quantitative methods for its analysis. In this lecture I would like to review some selective parts of this work that range from the early sixties up to now. I will then review some specific aspects of oxysteroids: enzymatic and non-enzymatic formation, how they can be used as markers for different pathological states, role of oxysterols as regulators of cholesterol homeostasis, methodology aspects.

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2. 7α-Hydroxy-4-cholesten-3-one

This steroids had previously been synthesized in 1961 by my tutor, Henry Danielsson, by a synthetic route from cholesteryl benzoate and with a yield of only 0.2%. A small amount of tritium labelled material had been prepared and injected in a bile fistula rat. In contrast to the expectations, only small amounts of the normal bile acids cholic acid and chenodeoxycholic acid were formed from the labelled steroid. Henry Danielsson believed, however, that the low yield may be due to the fact that the injected steroid had a very low specific radioactivity and that unphysiologically high levels had been injected. My first project was therefore to try to synthesize 7α-hydroxy-4-cholesten-3-one with very high specific radioactivity that would allow injections of only trace amounts of the steroid to bile fistula rats.

Because of the fact that I had to work on this project in parallel with my medical studies, it took me more than a year to find out a suitable synthetic procedure to get materials with high specific radioactivity. The crucial and critical step in the new synthetic procedure was the introduction of the delta 4 double bond by selenium dioxide. My new method of synthesis used chenodeoxycholic acid as starting material and the product could be obtained by only three separate steps with a total yield of about 5%. (Fig. 1). Since it was easy to label chenodeoxycholic acid with tritium it became possible to synthesize labelled 7α-hydroxy-4-cholesten-3-one with high specific radioactivity. When trace amounts of tritium labelled 7α-hydroxy-4-cholesten-3-one were injected into a bile fistula rat, there was a high yield of radioactive cholic acid and chenodeoxycholic acid. When the tritium labelled oxysterol was diluted with unlabelled oxysterol and injected into a bile fistula rat, a number of other radioactive bile acids were formed that could not be identified. This study was published in 1965 and became the first paper in my thesis that was completed 4 years later [1].

Thus we were convinced that 7α-hydroxy-4-cholesten-3-one is an intermediate in the conversion of cholesterol into bile acids. We could also show that this oxysterol is formed from 7α-hydroxycholesterol in microsomal preparations from rat liver. One of the challenges was to show if the double bond isomerizes from 5 to 4 position first or if the oxidation of the 3β-hydroxy group is the first step (Fig. 2). I could show convincingly that oxidation of the 3β-hydroxyl group is the first and rate-limiting step. The best evidence for this was my demonstration of an isotope effect in the oxidation of the 3β-hydroxyl group when the hydrogen atom at 3α was replaced with a deuterium or tritium atom [2]. Such an isotope effect is only possible if the carbon–hydrogen bond is broken in a rate-limiting step.

The problem was then to decide if two different enzymes are involved in the reaction: a 3β-hydroxysteroid dehydrogenase catalyzing oxidation of the 3β-hydroxy group and an isomerase catalyzing the conversion of the delta 5 double bond into the delta 4 double bond. Different attempts to isolate the postulated intermediate 7α-hydroxy-5-cholesten-3-one in the conversion failed, however. I also failed to synthesize the postulated intermediate in the conversion, and all attempts resulted in loss of the very labile 7α-hydroxyl group. I draw the conclusion that it was most likely that the dehydrogenase is able to catalyze both the oxidation of the 3β-hydroxy group and the isomerization. Thus the enzymatically formed intermediate may be protected from elimination of the 7α-hydroxyl group by association to a suitable group on the enzyme. I could also show that the enzymatic isomerization involved a transfer of hydrogen from the 4β- to the 6β-position.

There were still scientists, however, who believed that there must be a specific isomerase involved in the conversion of 7α-hydroxycholesterol into 7α-hydroxy-4-cholesten-3-one.

More than 20 years later, I became involved in a study demonstrating beyond all doubts that only one enzyme is involved in both the oxidation of the 3β-hydroxy group and the isomerization of the delta 5 double bond [3]. Expression cloning was used to isolate cDNAs encoding the microsomal 3β-hydroxy-delta-5-C27-steroid oxidoreductase. When this cDNA was transfected into cultured cells it encoded an enzyme that was active against a number of 7α-hydroxylated 3β-hydroxy-delta-5-steroids. This was convincing evidence for my suggestion that only one enzyme is required to catalyze both the oxidation and the isomerization.

The gene coding for the enzyme was shown to be mutated in a patient with urinary excretion of 3β-hydroxy-delta-5-bile acids. About 10 years earlier, we had shown that fibroblasts isolated from this patient had a reduced capacity to convert 7α-hydroxycholeserol into 7α-hydroxy-4-cholesten-3-one [4].

I would like to review the different metabolic fates of 7α-hydroxy-4-cholesten-3-one. As shown in Fig. 3 this compound can be 27-hydroxylated, 12α-hydroxylated, converted into the 5β- and 5α-saturated steroid and finally dehydrated.

In collaboration with a Norwegian group I had showed that the basal biochemical defect in patients with the rare inborn disease *Cerebrotendinous xanthomatosis* (CTX) is a defect sterol 27-hydroxylase [5]. This disease is characterized by development of cholestanol and cholesterol-containing xanthomas in the tendons and in the brain. It was difficult to understand the mechanism

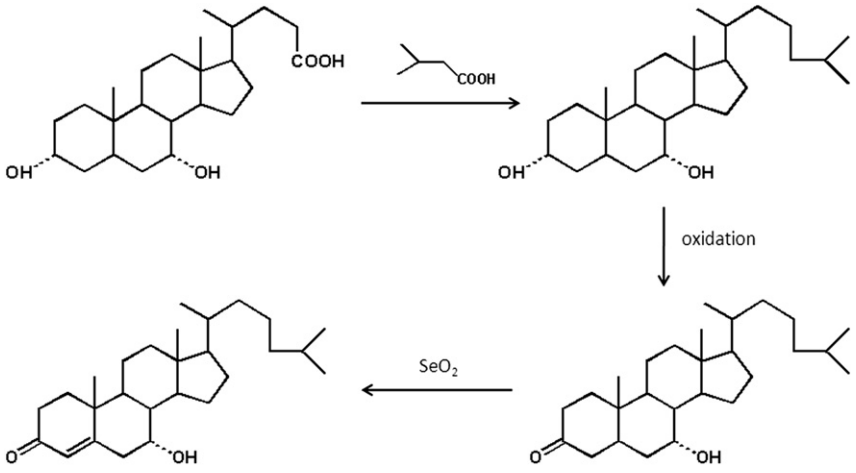

Fig. 1. Synthesis of 7α-hydroxy-4-cholesten-3-one.