Within the University of Washington School of Pharmacy, Associate Professor Nina Isoherranen’s research focuses upon the biochemical characterisation of the CYP26 enzymes and their role in retinoic acid metabolism.

Firstly, what are the central aims of the project? What do you hope to discover from this particular piece of research?

The central aim of the project is to establish the biological role of the CYP26 enzymes during childhood and adult life and to determine whether modulation of the activity of these enzymes, via inhibition or induction by xenobiotics, impacts human health. Our studies should also establish the potential value of individual CYP26 enzymes as drug targets in specific tissues. We aim to discover the role that the major enzymes in the CYP26 family play in regulating retinoid action and pharmacology, within specific tissues.

The CYP26 family includes three enzymes, CYP26A1, CYP26B1 and CYP26C1 that are very highly conserved in vertebrates (such as xenopus, as well as zebrafish, chicken, mice and humans). Despite large differences in the protein sequence between the CYP26 enzymes, all three enzymes appear to metabolise retinoic acid, the active metabolite of vitamin A.

What do you hypothesise is the mechanism of all-trans retinoic acid (atRA) depletion? In what way have you utilised transgenic mice studies to further understand this process?

We believe that the main mechanism of atRA depletion is via clearance by the CYP26 enzymes to hydroxylated products. However, some of these products may have actions of their own that remain to be discovered. Unfortunately the knock-out of CYP26A1 and CYP26B1 in embryonic mice is lethal and, as a result, we have not been able to use the knock-out mice for studying the importance of CYP26 enzymes after birth. However, we hope that in the future we will be able to use conditional knock-out mice for our studies.

Have you adopted any specialist techniques to complete the research?

We have developed a recombinant system to express the CYP26 enzymes. This process is necessary because it enables us to undertake biochemical and functional studies of these enzymes. We also use very sophisticated mass spectrometry approaches, in order to identify and detect atRA as well as the metabolic products formed by CYP26 enzymes. In addition, our research has required synthetic chemistry to generate the metabolites of atRA and selective inhibitors of CYP26 enzymes. The major gain in improving our understanding has been to put all of these techniques together with other collaborative efforts to better understand the enzyme function.

Are there any further applications of this research?

Retinoids are very effective in treating skin diseases, particularly psoriasis and acne. It is likely that the inhibition of CYP26 enzymes in the skin by novel compounds could improve therapy for topical treatments. Additionally, retinoids have been found to have potential utility in treatment of a multitude of diseases such as cancers and metabolic disease. Retinoids have also been suggested to hold promise in treatment of Alzheimer’s disease and be useful in immune system modulation. We hope that our studies on the basic function of CYP26 enzymes...
VITAMIN A IS important for growth and development. It can be obtained through foods rich in retinyl esters – the major form of vitamin A in animal-based foods – such as liver, meats, milk and eggs, and non-animal based foods rich in β-carotene – another form of vitamin A – including carrots and other yellow-orange vegetables. Both forms of vitamin A are metabolised in the body to create a very important metabolite, known as retinoic acid.

The reason for retinoic acid’s importance, and the scientific interest surrounding it, is its role within the human body – it is essential for a variety of normal biological functions. The better known effects of retinoic acid relate to its role in supporting growth in numerous animal species and modulating organogenesis and differentiation during foetal development. Furthermore, retinoic acid plays a role in neuronal differentiation, has been implicated in mediating lipid homeostasis and is also required for the maintenance of immunity. Indeed, the World Health Organization estimates that over 125 million children are vitamin A deficient and millions of deaths could be prevented annually with proper vitamin A nutriture. Due to its effects in immune cells, retinoic acid is also used in HIV vaccine development and is an approved drug for the treatment of acute promyelocytic leukaemia and Kaopsis sarcoma, as well as in neuroblastoma and acne.

Despite the multitude of benefits that retinoic acid boasts, an excess of retinoic acid can lead, in addition to teratogenicity, to bone pain, liver toxicity and various gastrointestinal (GI) effects. For this reason, by understanding how to control concentrations in target tissues, therapeutic advances can be made to treat these conditions. Therefore, a team of scientists from the University of Washington, led by Associate Professor Nina Isoherranen, is trying to understand whether certain substances impair normal retinoic acid homeostasis and vitamin A intake, and hence cause adverse effects. Their research will provide a much needed insight into the workings of this vital substance in the human body. An extension of their studies is to evaluate if specific increase in retinoic acid concentrations in select tissues can provide significant therapeutic benefits.

P450 ENZYMES

The overall objective of the team’s research project is to define the importance of CYP26A1 and CYP26B1, both of which are cytochrome P450 enzymes. Cytochrome P450 enzymes are heme proteins that generally carry out oxidative reactions, and are involved in the elimination of many drugs. P450 enzymes are also responsible for generating many of the steroids, as well as modulating signalling by lipid soluble vitamins. The team’s specific angle is to establish the role of CYP26A1 and CYP26B1 in maintaining cellular and circulating RA homeostasis during childhood and adult life. Furthermore, they hope to determine how xenobiotic-CYP26 interactions alter retinoic acid isomer concentrations in specific target tissues and in serum.

To put this in context, CYP26 enzymes were discovered through genomic screening (microarrays) of retinoic acid induced proteins in regenerating zebrafish fins. It is only subsequently that genetic screening has identified the human homologs of the same enzymes. Yet in spite of this discovery, the role of hepatic and extrahepatic CYP26 enzymes is not well established. What the team has...
ROLE OF CYP26A1 AND CYP26B1 IN MAINTAINING CELLULAR AND CIRCULATING RA HOMEOSTASIS

OBJECTIVES
The overall objective of the studies is to determine the role of CYP26A1 and CYP26B1 in regulating all-trans-retinoic acid levels in vivo, with the goal of understanding how modification of enzyme function (induction, inhibition or genetic polymorphisms) might affect retinoic acid homeostasis and an individual’s health. Research in the lab includes experiments that characterise the basic biochemistry of the CYP26 enzymes as well as the regulation of their expression and their pharmacogenetics.

KEY COLLABORATORS
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demonstrated so far, is that CYP26A1 is the main liver CYP26 enzyme and is responsible for retinoic acid clearance in the liver. Additionally, the project members think it likely that both CYP26A1 and CYP26B1 play important roles in regulating retinoic acid concentrations outside the liver. In this vein, they have detected the expression of both enzymes in many extrahepatic tissues and some clinical studies have shown that inhibition of CYP26 enzymes results in increased retinoic acid concentrations in serum.

TESTING HYPOTHESES
All-trans retinoic acid (atRA), the biologically active form of vitamin A, is essential for diverse biological functions and while the positive effects of atRA are well documented, the fact that they are concentration dependent is slightly less well understood. It is known that compounds which inhibit atRA metabolism can provide great therapeutic benefits in specific diseases by increasing cellular and circulating atRA concentrations, but if ATRA concentrations are increased too high the inhibition of ATRA metabolism impairs its beneficial effects on individual’s health. However the reasons surrounding this are not well founded.

While substantial progress has been made in our understanding of retinoid metabolism and function, the mechanisms that control circulating and tissue atRA concentrations in humans are still uncertain. This is precisely the knowledge-gap that the Washington team seeks to fill. In particular, there is very limited knowledge on how xenobiotics affect ATRA homeostasis and whether xenobiotic-ATRA interactions cause toxicity or can be used for drug therapy. With a desire to learn more about these phenomena, the team has been working to test their hypotheses that hepatic and extrapleptic CYP26 enzymes are responsible for ATRA clearance and that the selective inhibition of CYP26A1 and/or CYP26B1 will increase circulating and tissue ATRA concentrations resulting in fine-tuning of atRA concentrations in tissue specific manner. The specific aims of the research are all designed to establish a comprehensive understanding of tissue specific clearance of atRA, its contribution to atRA homeostasis and its susceptibility to inhibition by drugs and other xenobiotics. In order to draw conclusions on this, the researchers will characterise the importance of the CYP26 enzymes in clearance of RA isomers and metabolites.

As well as finding the answers to their specific questions, the result of the studies could also provide important information of CYP26 enzymes in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could prove groundbreaking in terms of drug targets and regulators of disease progression. Such information could provide great therapeutic benefits in specific diseases by increasing cellular and circulating atRA concentrations, but if ATRA concentrations are increased too high the inhibition of ATRA metabolism impairs its beneficial effects on individual’s health. However the reasons surrounding this are not well founded.

MEDICAL PROGRESS
Retinoids have already shown promise in treating some cancers and are approved therapy for neuroblastoma, acute promyelocytic leukemia (APL) and Kaposis sarcoma. Resistance to retinoid therapy in APL patients is generally believed to be due to CYP26A1 induction. Resistance to retinoids in other cancers has also been suggested to be a result of increased metabolism of retinoic acid in cancer cells and/or in the liver. Therefore, better understanding of the structure-function of the CYP26 enzymes is vital, as it could allow development of compounds that can be given together with retinoic acid to improve patient outcomes and combat resistance.

There also seems to be a link between late onset Alzheimer’s disease and defective retinoid signalling. To date, clinical studies have found that treatment with retinoic acid improves memory and cognitive function. Retinoic acid is also known to stimulate neuronal differentiation and regulate immune responses that potentially play a role in Alzheimer’s. Hence, increasing retinoic acid levels in the brain may be beneficial in its treatment. This could be obtained either via treatment with a synthetic retinoid or with inhibiting retinoic acid clearance that would increase endogenous concentrations of retinoic acid. The team in Washington hopes to better establish the role of CYP26 enzymes in regulating retinoic acid concentrations in the brain, so to further elucidate upon the potential for medical improvements.

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