

Gut feelings on reactive oxygen species

Attendees at UCD's Charles Institute Seminar Series heard a presentation on the clinical importance of reactive oxygen species and the complex interactive relationship between the gut and skin

The Charles Institute, Ireland's national dermatology research and education centre, played host to a range of guest speakers who covered a variety of topics ranging from skin cancer to psoriasis, among others. The series, which was sponsored by RELIFE (part of the A.Menarini group), was designed to provide expert practical advice from a range of distinguished national and international experts in their respective fields and was chaired by Prof Desmond Tobin, Professor of Dermatological Science at UCD School of Medicine and Director of the Charles Institute of Dermatology. Each seminar in the series was broadcast to attendees with a special interest in dermatology in other locations, who accessed each talk remotely via an audio-visual link.

Attendees at the seminar series heard a presentation from Prof Ulla Knaus, Professor of Immunobiology at the Conway Institute, School of Medicine, University College Dublin, on the topic of 'Reactive Oxygen Species — Lessons from Gut to Skin'.

Prof Knaus presented the attendees with an overview of research into reactive oxygen and cellular responses: "Everything starts with oxygen, but there are a lot of other products and conversion products," she told the seminar. "In the cells, there is constant conversion and degradation but when you look at the gut, it all becomes much more complex because in the gut, you have many more 'players'. These players don't only come from the host — they come from the microbiota, so they can be generated and used for signalling from the bacteria and from the host." One example of this is in inflammation, where the host will produce more nitrate and this can be used as an electron receptor by certain bacteria, she explained, "for example, pathogenic E.Coli and proteobacteria, and they can overgrow," said Prof Knaus. "That's what you see in, for example, inflammatory bowel disease (IBD), where you have an overgrowth in dysbiosis by proteobacteria." It is important to remember that all of these elements are interconnected, Prof Knaus pointed out.

She presented further research to illustrate these points. "Those of us in the field of reactive oxygen species don't really like the term 'oxidative stress'; I personally think that in general, reactive oxygen species are generally good and it's really rare that they are not."

Dermatology

Prof Knaus touched on the significance of this research for dermatology, focusing on three different areas to shed light on the interaction between the gut and the skin. "These areas are migration, which is relevant to wound-healing and potentially metastases; the antimicrobial host, which is also potentially important in

wound-healing; and also inflammatory disease," she pointed out. "If you look at migration... in studies from 2000, they only had a fluorescence indicator for hydrogen peroxide, called DCFDA. Some people still use it now but it is very artefact-prone... if you look closer at these migrating cells, you will see that there is ROS production in the leading edge, directly in the ruffle, where the cell starts to go."

Prof Knaus also addressed the importance of carefully examining the dose of hydrogen peroxide that should be used and at what point the dose becomes detrimental. Again drawing the attendees' attention to previous research, she said: "What the research found was that if you have a low concentration of hydrogen peroxide on a wound, you get much faster wound closure. If you use a high concentration, then you have much slower wound closure, so the efficacy depends on the concentration, and that doesn't just apply to hydrogen peroxide," said Prof Knaus.

"Then, if you look at the gut, there are certain soluble agonists secreted from lactobacilli that will activate NOX1 in the colon barrier and this will lead to redox signalling to kinases and also to the cytoskeleton, altering and enhancing cell proliferation and cell migration, which leads to faster wound closure," she continued.

Melanoma

Prof Knaus also touched briefly on mechanisms of antioxidants in melanoma and told the attendees: "A lot of the details are not known here and there is not much literature covering it, but you will always see that NAH oxidase NOX4 is highly up-regulated in melanomas," she explained. "We still don't know exactly what it is doing there but what we do know is that taking out redox signalling here is very bad — it doesn't really matter for melanoma growth... but there is a huge change in the metastatic burden and the number of melanoma cells is vastly increased if you use antioxidants. This has been shown, not only in melanoma, but also in lung cancer."

Prof Knaus presented patient data on intestinal inflammation and said that until now, there had not been found a gain in function mutations in NADPH oxidase or in any ROS-producing enzyme. "That doesn't mean they don't exist; they have just not been identified," Prof Knaus told the seminar. "But there are loss of function mutations in NADPH oxidase. There are some that have been known for some years now that are important in hyperthyroidism and there are also some that have been known for years in chronic granulomatous diseases (CGD), which are life-threatening, inherited conditions due to variants in the NOX2 complex. But there are also new variants," she explained. "These are variants in the NOX2 complex that are

not CGD phenotype, so they produce too much ROS — these patients will not have CGD, but they will have inflammatory bowel disease that is called very early-onset IBD and occurs in children under the age of six years."

Prof Knaus and her colleagues have also identified mutations in NOX1 and DUOX2 that lead to a highly-increased risk of very early-onset IBD, she explained. "We think there are many patients who have a problem with this because there are many of the variants that have been shown to be a risk factor for IBD upstream of NADPH oxidases and all of them impinge on oxidase signalling, so we think ROS production is reduced in these patients."

Ageing

During a lively, interactive Q&A session Prof Tobin raised the question of the observed reduction of antioxidant enzyme expression and its activity associated with ageing. If people lose antioxidative stress protection with age, could this result in a functional deficiency, or might the skin microbiome act as a compensatory factor in maintaining optimal functioning, he asked.

"Researchers have looked at this possibility in ageing generally, but to my knowledge, not specifically in terms of skin," Prof Knaus replied. "Also, there is less ROS production with ageing, unless the person is very active. It also depends on which enzymes you are looking at, because a lot of the time, there might be changes in the mitochondrial functioning, which is a very different thing. We believe that the mitochondria — specifically, if they are not working properly anymore — have much higher electron leaks. If you have more electron leaks, you will have more suboxide production," she explained.

"The innate immune system with higher age does not really work very well, so you would expect less ROS production but, for example, mitochondria could very well be damaged in a certain way and that could create a completely different picture. But even in that case, you have to think about what's happening because with mitochondria, one of the problems you have is iron in the mitochondria. So, if you use hydrogen peroxide and make enzymes within the mitochondria, you could create more damage, because you can cause hydroxyl radicals. If that is the case then yes, I agree with that hypothesis but even then, you are not actually fluctuating any more — you are 'stuck' and you won't be able to achieve normal homeostasis."

Lactobacilli

Prof Knaus also answered a question relating to lactobacilli/hydrogen peroxide combinations and whether these can be beneficial in the early onset of irritable bowel disease. She was also asked wheth-



Prof Ulla Knaus

er data have been compelling enough to move this research area to the translational stage in the clinic, and Prof Knaus responded: "There have been lots of trials involving lactobacilli as a probiotic in humans. In some trials, it would appear that as a combination, it is more beneficial than if it is a single one of these.

"But the real problem with lactobacilli in humans is, in the past 150 years or so, we have lost our ability to have a more-or-less stable colonisation of lactobacilli," she continued. "Most of the probiotics we eat actually just go straight through our system. They never settle in the body and if they can't settle, they can't actually do anything. It's an even bigger problem if you have inflammation in the gut, because that environment is so toxic for lactobacilli that they don't settle at all. Very often, there is no mucous layer anymore, and there are nitrates, which they don't like either, so it is very difficult to get hydrogen peroxide into the gut in this way."

Speaking with the *Medical Independent (MI)* following her presentation, Prof Knaus said the area of research examining the relationship between the gut and other aspects of physical health is an exciting field that could transform our current understanding of patient care. "We are very lucky and happy to be working in this area of research," she said. "Particularly here in UCD, I have the opportunity to interact with clinicians a lot more [than in previous positions], and that is one of the main reasons I came to work here.

"Clearly, my colleagues use other cellular models too but I believe what is important is to understand that this is a very complex area," she told *MI*. "We don't look for very easy, fast solutions — we need a thorough understanding and I believe that from that understanding, therapies will emerge that will be very beneficial for mankind. I strongly believe that. But if there was one simple take-away message from today, it is that we need to be very careful when using antioxidants."

RELIFE has had no input into the content of this article or series of seminars.