Impact Objectives

- Further investigate the potential of polymethoxy flavonoids (PMFs) as a means of halting the recruitment of osteoclasts in periodontal disease
- Use rat models to test the effectiveness of sudachitin
- Verify the safety of sudachitin and work towards the development of a toothpaste containing sudachitin as a potential preventative measure against periodontal disease

Reducing reactive oxygen species (ROS) in gum disease

***Dr Junta Ito** here talks about his research into periodontal disease and how he may have found a compound capable of preventing it*

How did you come to work in this area of dentistry? What interests you most about this field?

My passion for my research arises from the simple questions: Why do people eat? and What is the meaning of eating? I believe in a well-managed diet. However, even if the patient achieves a well-balanced diet, if the tooth is not healthy, you will not be able to eat the meal. I pursued a career in the dental field to attempt to tackle the disparity between a healthy diet and healthy teeth. Within this rather broad field, I am most interested in the maintenance of the alveolar bone that supports the teeth and how this can be enhanced through the use of functional ingredients found in food. My key research interests are bone metabolism, anatomy and sports nutrition.

Could you explain in simple terms what alveolar bone reabsorption means and its significance?

Alveolar bone is the most important tissue of all the dental support tissues. This is because the alveolar bone directly supports the teeth. When the alveolar bone is destroyed by periodontal disease, it can no longer support the tooth and it will eventually be lost. Naturally, the loss of teeth makes eating, pronunciation and vocalisation difficult, and the patient's quality of life significantly and suddenly decreases.

How does reactive oxygen species (ROS) affect bone metabolism?

We hypothesise that reactive oxygen species (ROS) stimulate osteoclast differentiation. In other words, it is thought that a local increase in ROS amongst alveolar bone affected by periodontal disease induces differentiation and activation of osteoclasts. Osteoclasts are cells that break down bone tissue, which includes the alveolar bone in this context. Therefore, we believe limiting the overproduction of ROS in osteoclasts through the action of sudachitin could prevent extreme formation of osteoclasts induced by local inflammation and prevent alveolar bone destruction.

What led you to work with sudachitin? Why did you feel this would have an effect on osteoclast formation?

I devised this research when I learned that polymethoxy flavonoids (PMF) influence bone metabolism in other researchers' papers. It seemed this could provide an obvious benefit in my field of dentistry. I started by exploring the powerful PMFs in fruits native to Japan. The next step is turning sudachitin into a viable treatment for periodontal disease in our rat model. This should establish whether or not we would be able to use sudachitin to prevent periodontal disease in people. Ideally, I would like to apply it to people in the near future. If we can verify the safety of sudachitin, we would like to develop toothpaste containing sudachitin for periodontal disease prevention.

What other fruits believed to contain PMFs are you planning to investigate?

PMFs are found in high concentrations in citrus fruits. It has also been linked to being part of a healthy diet. We are investigating two PMFs of particular interest in Japan: nobiletin from the extremely common ponkan fruit and tangeretin from citrus depressa. These PMFs have already been shown to inhibit bone loss reduction using periodontitis in a mouse model. I think it would be tricky to uncover completely novel PMFs as they are now well characterised, but it's an interesting challenge and I always keep my eyes open!

How important is collaboration to the success of your work and what are each of your collaborators bringing to the table?

Throughout my research career, discussion with collaborators has always been indispensable. For this particular project, the discussions and consultations with Professor Yoshiyuki Hakeda of Meikai University School of Dentistry, Japan, were and are very important. He brings expertise in molecular biology and has taught me how to think about and process problems in my work. My research could not have been successful without him. ▶

Preventing periodontal disease

*Researchers at Japan's **Josai University** and **Meikai University School of Dentistry**, are uncovering a novel method in the prevention of periodontal disease, one of the most prevalent and challenging oral diseases*

Eating is essential to every human being. It is the sole way we ingest the energy and nutrients we need to survive. Our teeth play a huge role in this process, as the majority of food is solid and requires physically breaking into small chunks. The great irony of the process is that, even if a person follows an extremely healthy diet, their teeth can still be damaged by the eating process. A variety of afflictions can affect the teeth, such as tooth decay and periodontal disease. If left untreated, these diseases will eventually lead to teeth falling out and a significant diminishment in a person's quality of life. Not only will eating solid food be difficult, but also even basic communication can become complicated

As with many areas of medicine, dentistry looks to both prevent and treat. Typically, prevention takes the form of regular teeth brushing and other oral hygiene techniques such as flossing and mouthwash. Prevention also takes the form of encouraging patients to avoid the food and drinks that will have the biggest negative impact. Treatments are broad depending on the disease, but typically aim to remove the diseased part and repair or replace it. Ideally, however, a great variety of preventative measures could be available with which to reduce a person's chances of developing all the types of oral disease.

Dr Junta Ito of Josai University Faculty of Pharmaceutical Science, Japan, is working on a novel preventative method to tackle periodontal disease. Periodontal or gum disease is usually caused by bacteria and manifests as an on-going inflammation of the gums and bone around the teeth. As this process advances, the gums recede and the teeth become gradually more exposed. At the same time, the bone tissue holding the teeth in place is also being destroyed. Eventually, this leads to the teeth falling out without any support holding them in place. Around 568 million people are affected by the disease, with an estimated 50 per cent of over 30s afflicted and 70 per cent of over 70s. There are various risk factors that increase the chances and the usual preventative dental measures are helpful, however, sometimes there is no preventing the disease. Ito has discovered a natural compound that seems capable of preventing the degradation of the bone seen in periodontal disease.

BONE REABSORPTION

Part of the underlying cause of the bone recession characterising periodontal disease is due to the action of osteoclasts. These are important cells for the regulation and maintenance of all types of bone. They are primarily responsible for breaking down specific areas of bone and the reabsorption of the contents into circulation. This is typically necessary for several reasons. Firstly, it helps to maintain the correct bone structure and stops bone growing into inappropriate places or shapes. Secondly, it is crucial for healing when bones are damaged. Like maintenance, it is essential for healing to be able to control the process and remove bone that is in the wrong place. Osteoclasts, therefore, are extremely important to healthy bones. It is not surprising that osteoclasts are also responsible for the maintenance of healthy alveolar bone.

However, as with many chronic diseases, the osteoclast's role in healing and maintenance becomes dysregulated in periodontal disease. The persistent infections associated with this disease means that the infected area – ie, the gums and alveolar bone – are chronically inflamed. This inflammation, in turn, causes osteoclasts to be differentiated, recruited and maintained in the area. It is the long-term presence of the osteoclasts that leads to the diminishment of the alveolar bone through the reabsorption process. Ito's work has focused on blocking the recruitment of osteoclasts to alveolar bone – osteoclastogenesis. If osteoclasts can be blocked in this specific, local level, periodontal disease can be halted early and the teeth can be saved.

SUPER SUDACHITIN

Ito's research has centred on polymethoxy flavonoids (PMFs) as a potential group of compounds capable of halting the recruitment of osteoclasts in periodontal disease. These chemicals are found naturally occurring in many citrus fruits. The compounds are potent anti-oxidants capable of absorbing and dissipating the harmful effects of reactive oxygen species (ROS). ROS are both found in the environment and are by-products of the normal cellular metabolism. They are found at high concentrations at sites of injury or infection,

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as they are also a method of immune killing. Additionally, they act as a signal for certain cells to be differentiated and recruited at particular sites. Cells made in this way include osteoclasts, as they are generally required at sites of injury and infection. Ito is proposing to use the most effective PMFs to reduce ROS in the gums and alveolar bone to ultimately stop the differentiation and recruitment of osteoclasts to that area.

Following this aim, Ito has screened many PMFs in many cell line models and has settled on sudachitin. This molecule is found in the sudachi citrus fruit famous in Japan for its unique acidic flavouring. Ito and his team started by testing the molecule on cell lines containing the precursors to osteoclasts. These can be forced to differentiate into osteoclasts in the presence of lipopolysaccharide, which induces inflammation. Inflammation creates high levels of ROS in the cells, one of the key signals in differentiation. However, with the addition of sudachitin, the cells did not show the same differentiation pattern. Indeed, cell cultures with sudachitin did not differentiate into osteoclasts at all. Instead, they stayed in the progenitor form and therefore cannot involve themselves in bone reabsorption. Crucially, sudachitin has no effect on the viability of cells. This means the cells are stopped from differentiating, but not killed or damaged in any way. This is an essential property if sudachitin is to be employed as a preventative treatment.

These results are extremely promising, but the next step in the investigation is to test sudachitin in a rat model. Ito and his team will use a particular model that aims to replicate periodontal disease in an accelerated manner in rats. This model induces inflammation in the site of the disease, allowing the effects of adding sudachitin to be examined. Ito explains the process behind this: 'The first step in the administration plan of sudachitin is to confirm the preventive effect of periodontal disease by local administration of sudachitin. The second step confirms the preventive effect of periodontitis by sustained oral ingestion of sudachitin.' As well as monitoring the effects of sudachitin on periodontal disease, these experiments should establish that there are no off-target negative effects to regular sudachitin administration.

PREVENTATIVE MEASURES

Ito's research suggests a novel method of reducing this widespread and difficult-to-treat dental disease. If the rat model shows sudachitin to be effective, Ito is looking to establish the compound as a new component of toothpaste. With regular application, sudachitin would then be able to drastically slow and potentially even eliminate periodontal disease in those who treat their teeth with care. In one stroke, this would eliminate one of the most pervasive and troublesome oral diseases. ●

Project Insights

FUNDING

This project is supported by Grant-in-Aid for Scientific Research Grant-in-Aid for Young Scientists (B).

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