Impact Objectives

- Delve deeper into the range of environmental factors affecting the sense of smell, including age, inflammation and cigarette smoke
- Understand the molecular mechanism of the pathology and the impact of upper respiratory tract inflammation as a result of environmental factors such as smoking
- Eventually devise methods to treat olfactory impairment through the use of easier and cheaper methods such as nose drop (collunarium) and oral supplements

Effects of smoking and ageing on olfaction and olfactory neurogenesis

__Assistant Professor Rumi Ueha__ from the __University of Tokyo__, Japan, and her colleagues __Professors Satoshi Ueha, Toshihiro Ito and Tatsuya Yamasoba__, discuss their project to investigate the effects of smoking and ageing on olfaction and olfactory neurogenesis

Assistant Professor Rumi Ueha

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Professor Toshihiro Ito

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Could you expand on your work focusing on ageing on the olfactory cell line?

We demonstrated a decline in the division and differentiation of neuro-progenitor cells and a decline in the number of mature olfactory receptor neurons (ORNs) associated with the decline in olfactory function related to ageing. ORNs are complex ligand-gate channels that, as a result of a significant number of receptor variants, enable the differentiation of different odours by the depolarisation of receptors on contact with different molecular compositions. The mechanism underlying this decline in ORNs involves the upregulation of inflammatory cytokines and a decrease in the expression of Igf-1 and tissue structure-related genes in the nasal mucosa. Considering our results, inflammatory cytokine inhibitors, insulin-like growth factor-1 (IGF-1) promoters and supplements, and activators of IGF-1 signal transduction, may be important clinical targets for preventing the decline in olfactory function associated with ageing.

Could you explain the processes involved in your project?

In the research we used a comprehensive microarray-based analysis of gene expression in the nasal mucosa to investigate the molecular mechanisms underlying ageing-associated reduction of ORNs. The method of research uses three distinct stages; the first is the production of the animal model and tissue collection followed by the verification of a number of cells and preparation of the coronal section of the olfactory epithelium. Following the measurement of the number of cells by immunohistochemical staining, the final stage of the research is to assess olfactory cell behaviour and the reaction to odours by an olfactory habituation/dishabituation test, and by measuring levels of a number of inflammatory cytokines by extraction of mRNA and DNA analysis and the level of IL-1β and IL-6 expression by QRT-PCR.

What results are you able to discuss?

Firstly, the results showed that cigarette smoke solution (CSS) impaired the olfactory progenitor cells and induced olfactory dysfunction in mice. Secondly, the results showed that the intranasal administration of CSS suppressed the recovery of ORNs and olfaction following methimazole-induced olfactory epithelial (OE) injury. Cigarette smoke solution administration decreases Igfi gene and IGF-1 protein expression in the injured-OE, and recombinant human IGF-I administration reverses CSS-induced suppression of ORN recovery. The results found that ORN recovery was not linked to changes in $SOX2^+$ ORN progenitor cells in the basal layer of the OE, but was linked to the impaired dividing of ORN progenitors and impaired increase in the number of $GAP43^+$ immature ORNs. At the molecular level, the data showed that in the nasal mucosa, mRNA expression levels of neurotrophic factors such as brain-derived neurotrophic factor, neurotrophin-3, neurotrophin-5, glial cell-derived neurotrophic factor and insulin-like growth factor-1 (IGF-1) were increased following OE injury. In addition, in the aged mice, it was also demonstrated that smoking reduced the number of mature ORNs and olfactory dysfunction by increasing ORN death in the OE, which eventually overwhelms the regenerative capacity of the epithelium.

Ageing and olfactory disorders

The '__Effects of smoking and ageing on olfaction and olfactory neurogenesis__' project is working to develop a greater understanding of how smoking in Japan influences olfactory disorders and how they could be treated

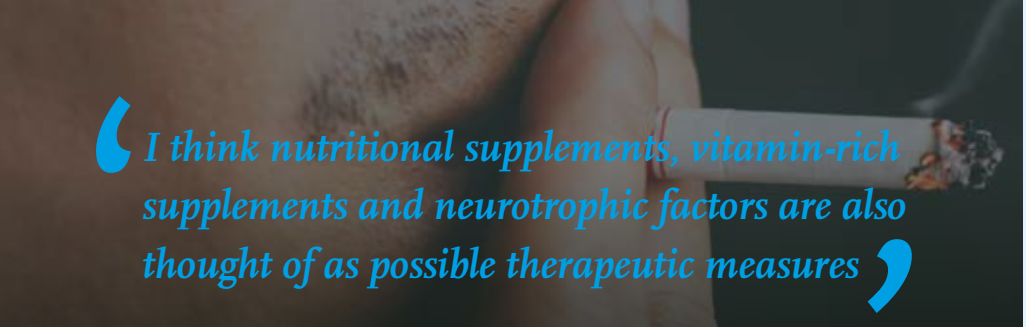
Our sense of smell is directly connected to survival and wellbeing. In Japan, the number of patients with olfactory disorder has increased, but prevention and treatment protocols remain limited. Research by Assistant Professor Rumi Ueha takes into consideration a range of environmental factors affecting the sense of smell, including age, inflammation and cigarette smoke. She highlights: 'Significant oxidation occurs to human beings exposed to daily life-creating stress and chronic rhinitis in the nasal region, and smoking is involved in the onset and deterioration of sinusitis.' Risk factors for the onset of chronic nasal and secondary nasal disorders affect sense of smell by changing the flow of air that exacerbates mucous membrane inflammation of the cavity and makes the passage of air more difficult, resulting in both breathing and olfactory disorders. Olfactory disorders can occur as a result of a variety of factors ranging from ageing to toxic chemical exposure, airway allergies, upper-airway viral infections, head trauma or development of neurodegenerative diseases, and in most cases loss of olfactory senses is a characteristic symptom. With an ageing population and increasing incidences of respiratory infections and asthma, as well as allergy related illnesses such as rhinitis, it is becoming increasingly important to understand the molecular mechanism of the pathology and understand the impact of upper respiratory tract infections as a result of environmental factors such as smoking. Ueha outlines three key objectives to her research: 'The objectives are to understand at the molecular level the effect of cigarette smoke on olfactory mechanisms, to assess how smoking can influence olfactory epithelium homeostasis and epithelial disorder recovery process, and determine how the age of patients can influence inflammation and recovery.' The analysis will focus on histological analysis of changes to cells at the molecular level.

MOLECULAR ANALYSIS OF CIGARETTE SMOKE IMPACT

In order to understand the impact of cigarette smoke at both the molecular and behavioural level, cigarette smoke solution (CSS) was administered periodically to mice prior to tissue extraction and molecular analysis were monitored in behavioural assessments. Ueha outlines: 'We administered CSS to mice over 24 days and then examined ORN (olfactory receptor neurons) existence, cell survival, inflammatory cytokine levels in the olfactory epithelium (OE), and olfaction using histological analyses, gene analyses and olfactory habituation/dishabituation testing.' In terms of molecular assessment, Ueha details: 'Olfactory marker protein-positive (OMP^+) ORN levels were then quantified and the number of $SOX2^+$ ORN progenitors, $GAP43^+$ immature ORNs, $Ki67^+$ cells, and cleaved $Cas3^+$ apoptotic cells per mm of basal layer length were manually counted using digital imaging analysis.' This molecular analysis was selected as olfactory marker proteins are expressed specifically in mature ORNs and Ki67 proteins are also a known cellular marker linked to cell proliferation and can be detected throughout the depth of the olfactory epithelium, mainly in the basal layer. In addition, Ueha says: 'Caspases are also crucial mediators of programmed cell death (apoptosis), and Cas3 is a major death protease activated during apoptotic processes, catalysing the specific cleavage of many key cellular proteins. As a result, it is important to determine rates of decline in olfactory receptors following exposure to cigarette smoke.' The research also recorded the time sensitivity of potential treatment intervention as the length of exposure to CSS is critical to the number of mature ORNs that do not recover after cessation of treatment resulting in persistent olfactory dysfunction only in the aged mice. As Ueha explains: 'We first examined the effect of continuous intranasal administration of CSS on ORNs in the young mice. We then examined the effects in the aged mice. In the young mice, the reduction in mature ORN numbers became more severe on day seven after the final intranasal administration of CSS, but then gradually recovered during days 14 to 28. In addition, olfaction also recovered within 14 days after the final intranasal administration of CSS. But, in the aged mice, the number of mature ORNs and olfaction did not recover with time.'

IGF1 REGULATION AND ROLE IN OLFACTORY DISORDERS

One of the key findings of the research is related to the first demonstration of a low-dose rhIGF-1 administration increasing the number of OMP^+ mature ORNs in the aged OE, although in comparison, a higher rhIGF-1 dose did not correspondingly have positive effects on the aged OE. Ueha comments: 'These dose-dependent effects of rhIGF-1 on the aged OE may be important for developing treatments for ageing-related olfactory dysfunction as IGF-1 is a growth factor that exerts trophic effects on neuronal development and regeneration.' The increase in the number of OMP+ mature ORNs after IGF-1 administration can be partly explained by the overwhelmingly higher cell proliferation of olfactory progenitors and immature ORNs compared to the increase in



'I think nutritional supplements, vitamin-rich supplements and neurotrophic factors are also thought of as possible therapeutic measures'

Cas3+ apoptotic cells. Ueha also recognises that: 'Our results indicate that the number of olfactory progenitors and immature and mature ORNs rises only by low-dose rhIGF-1 treatment and that high-dose rhIGF-1 administration did not increase the number of ORN progenitors and only promoted both proliferation and apoptosis of ORN progenitors/immature ORNs in the aged OE.' As it is the low-dose (not high-dose) rhIGF-1 administration that increases the number of OMP+ mature ORNs in the aged OE, this will affect dose levels and intervals for IGF administration, which is a key factor in treatments for ageing-related olfactory dysfunction and as Ueha acknowledges, requires further research. She says: 'The reduction in mature ORN numbers is associated with olfactory impairment in older populations and IGF-1 administered at an appropriate dose could prevent ageing-induced negative effects on ORNs. However, Ueha admits: 'Because our results were only obtained from mouse studies, work needs to focus on developing IGF-1 application for the treatment of human olfactory impairment.'

PREVENTING DYSOSMIA IN THE FUTURE

The results of this study into the impact of olfactory disorders has recognised the long-term impact of smoking. As Ueha explains: 'Not only the mature olfactory cells are impaired, but the olfactory epithelium progenitor cells are also impaired at the beginning.' She adds: 'Olfactory progenitor cells are impaired by the effect on olfactory cells by continuous exposure to smoke and there is an increase in the effects of the olfactory epithelium changes in olfactory disorders and the inflammatory response.' The research demonstrates how neuronal cells did not recover and cell death is increased and there is a strong differentiation in the ability of olfactory cells to regenerate and illness persists. 'In particular, the ability of epithelial cells to recover and regenerate reduces after prolonged exposure to tobacco smoke and in older people this can determine the severity of subsequent olfactory disorders,' Ueha explains. Ageing is associated with increasing levels of inflammatory activity and proinflammatory circulation of cytokines such as IL-1 and TNF, and as a result, impacts are increased in older people as the regenerative capacity of the olfactory epithelium is overwhelmed. Ueha underlines: 'In younger people, olfactory cell inflammation response is addressed the same way as any other cell inflammation and as a result, cell condition and response limits the short-term impact on health.' She adds: 'The research clarifies that delays to the recovery mechanism is a result of the division and differentiation process of olfactory progenitor cells and a failure to inhibit differentiation into mature olfactory cells is thought to determine the type and level of the olfactory disorder sustained as cell death is increased.' Ueha concludes: 'Whilst smoking should be deterred, there is the potential to develop mucosal receptor targeted treatment to reduce the rate of cell apoptosis and persistent olfactory disorders.'

FURTHER READING

For more information please visit the website: https://researchmap.jp/ruu/

RELEVANT PAPERS

1. Damage to Olfactory Progenitor Cells Is Involved in Cigarette Smoke-Induced Olfactory Dysfunction in Mice. *The American Journal of Pathology, doi: 10.1016/j.ajpath.2015.11.009*
2. Cigarette Smoke Delays Regeneration of the Olfactory Epithelium in Mice *Neurotoxicity research, doi: 10.1007/s12640-016-9617-5*
3. Reduction of Proliferating Olfactory Cells and Low Expression of Extracellular Matrix Genes Are Hallmarks of the Aged Olfactory Mucosa. *Frontiers in Aging Neuroscience, doi: 10.3389/fnagi.2018.00086*
4. Cigarette Smoke-Induced Cell Death Causes Persistent Olfactory Dysfunction in Aged Mice. *Frontiers in Aging Neuroscience, doi: 10.3389/fnagi.2018.00183*
5. Dose-Dependent Effects of Insulin-Like Growth Factor 1 in the Aged Olfactory Epithelium. *Frontiers in Aging Neuroscience, doi: 10.3389/fnagi.2018.00385*

Project Insights

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