Nevrargenics

Neuroprotection, neuroplasticity, and neurorepair

- Neurodegenerative diseases such as Alzheimer's are caused by permanent damage of the nerve cells and their connections.
- Unfortunately, there is no cure for such conditions yet.
- Retinoids can control important cell functions such as cell growth and division.
- Professor Andy Whiting, Nevrargenics, UK, has been investigating the different pathways through which retinoids act on nerve cells. His aim is to develop treatments that achieve the three N's: neuroprotection, neuroplasticity, and neurorepair.

Retinoids are molecules that are biochemically derived from vitamin A. Vitamin A comes from the food we eat and cannot be produced by our bodies. Inside the body, vitamin A is stored in the liver and converted to retinoic acid. There are several closely related variants of retinoic acid and collectively, these are called retinoids. Retinoids play many essential roles in the development of the body's organs, the regeneration of cells, the sense of vision, as well as the defence mechanisms of our body (immune system). One retinoid – trans-retinoic acid (ATRA) – is particularly important as it helps to control all stages of cell growth, development, and maturation.

The retinoids act by binding to special cell receptors called retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Predominantly, these are present in the cell's nucleus (the central part of the cell that controls its growth), though some RARs are expressed in the cytoplasm as well. In the nucleus, retinoids binding to their receptors activate chains of events – starting with DNA transcription and resulting in regulation of cell growth and tissue development (genomic effect). In the cytoplasm, however, retinoids interacting with their cell receptors have been shown to directly control the action of certain enzymes, such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), proteins that fine tune basic cell functions, including cell division and proliferation (non-genomic effect). These properties are a major part of the reason why retinoids have been used to date, to treat a number of conditions such as skin conditions, but also severe diseases such as breast and prostate cancer.

But what about nerves?

Retinoids play an important role in the development and ongoing protection of the central nervous system (CNS), as well as in the key repair processes of damaged nerve cells. They do this by stimulating cellular regeneration of their neurites (the projections of the cell body of a neuron that is necessary for the interaction between neurons and the function of our nervous system). Retinoids also contribute to the brain's constant requirement for changing and rewiring, vital for adapting to changing needs through external experiences and environmental cues. This process is called neuroplasticity and is necessary for learning, adapting, and creating new memories.

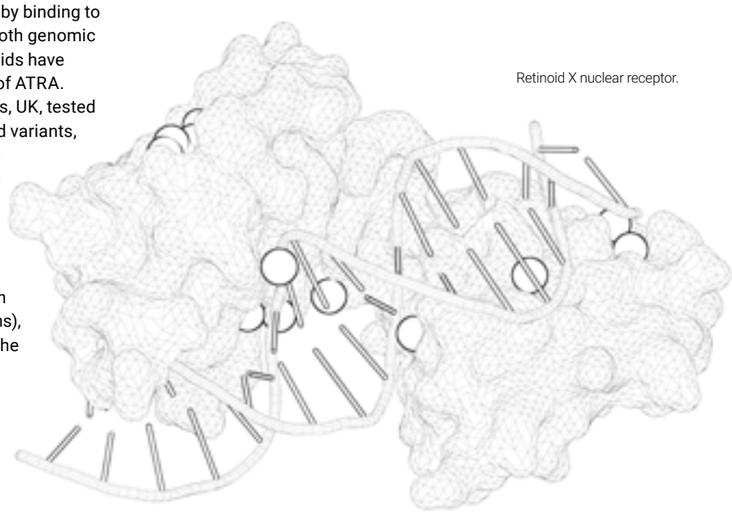
It has been shown that retinoids, including ATRA, work by binding to their nuclear and cytoplasmic cell receptors, through both genomic and non-genomic effects. A number of synthetic retinoids have been designed to have similar modes of action to that of ATRA. Professor Andy Whiting and colleagues at Nevrargenics, UK, tested a number of retinoids as well as their own novel retinoid variants, to find out more about their molecular structure versus function. The researchers were especially interested in understanding more about the potential dual action of retinoids, and their ensuing downstream actual effect on neurons. Nevrargenics' vision is to develop drugs that can treat neurodegenerative diseases such as Alzheimer's, by achieving the three N's: neuroprotection (to protect the structure and functionality of the neurons), neuroplasticity (as described above) and neurorepair (the regeneration of the nerve cells).

Testing the candidates

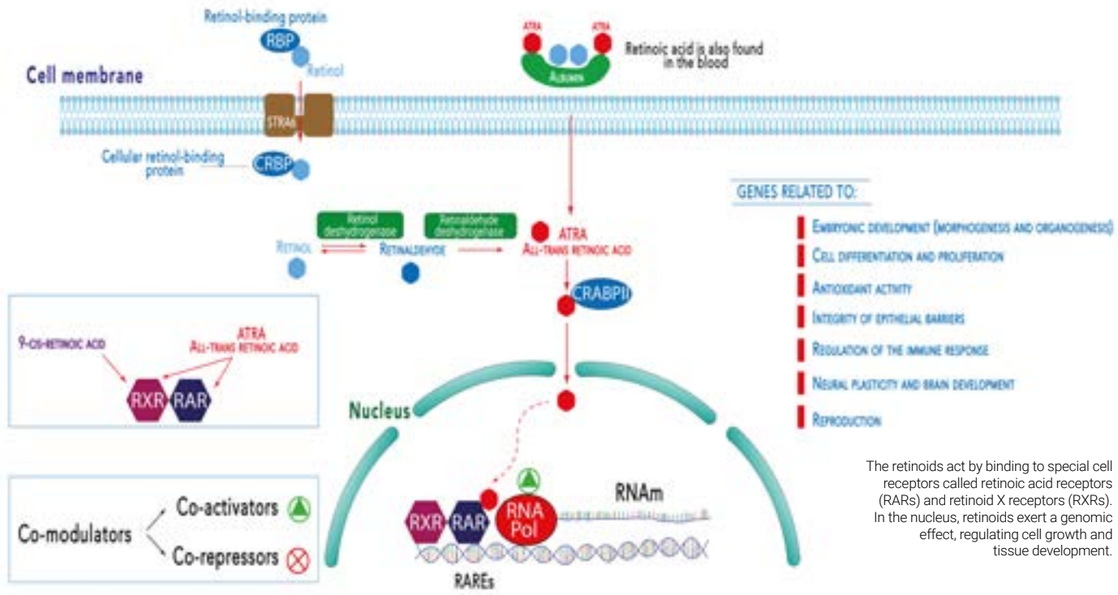
In one report, the team tested 28 novel synthetic retinoids, which structurally resemble many of the features of ATRA, and other retinoic acid variants. These agents were tested at various concentrations on Sil-15 cells, widely used laboratory cancer cells, and SH-SY5Y cells, another type of laboratory cancer cell originally derived from a nerve cell tumour (neuroblastoma).

Retinoids that were efficient at regulating both gene expression and enzyme activity directly increased the neurite length more than the others.

The researchers calculated the amount of RNA (molecules that are used by the cells as templates for the production of proteins) in the treated cells. The amount of RNA produced reflects the cell's protein building activity, which is triggered by the retinoids acting



Retinoid X nuclear receptor.



The retinoids act by binding to special cell receptors called retinoic acid receptors (RARs) and retinoid X receptors (RXRs). In the nucleus, retinoids exert a genomic effect, regulating cell growth and tissue development.

Yes, this is complex. But really complex disease mechanisms need really complex solutions.

The exceptionally high potency and improved physicochemical properties of these new drugs, compared to, for example ATRA, also means that lower concentrations are required for optimal treatment results, allowing for safer use with fewer side-effects. The researchers believe that their properties could make these small retinoids irreplaceable compounds of future therapeutic strategies against neurodegenerative disorders such as multiple sclerosis, Alzheimer's and Parkinsons disease, amylolateral sclerosis (ALS), and frontotemperal dementia (FTD).

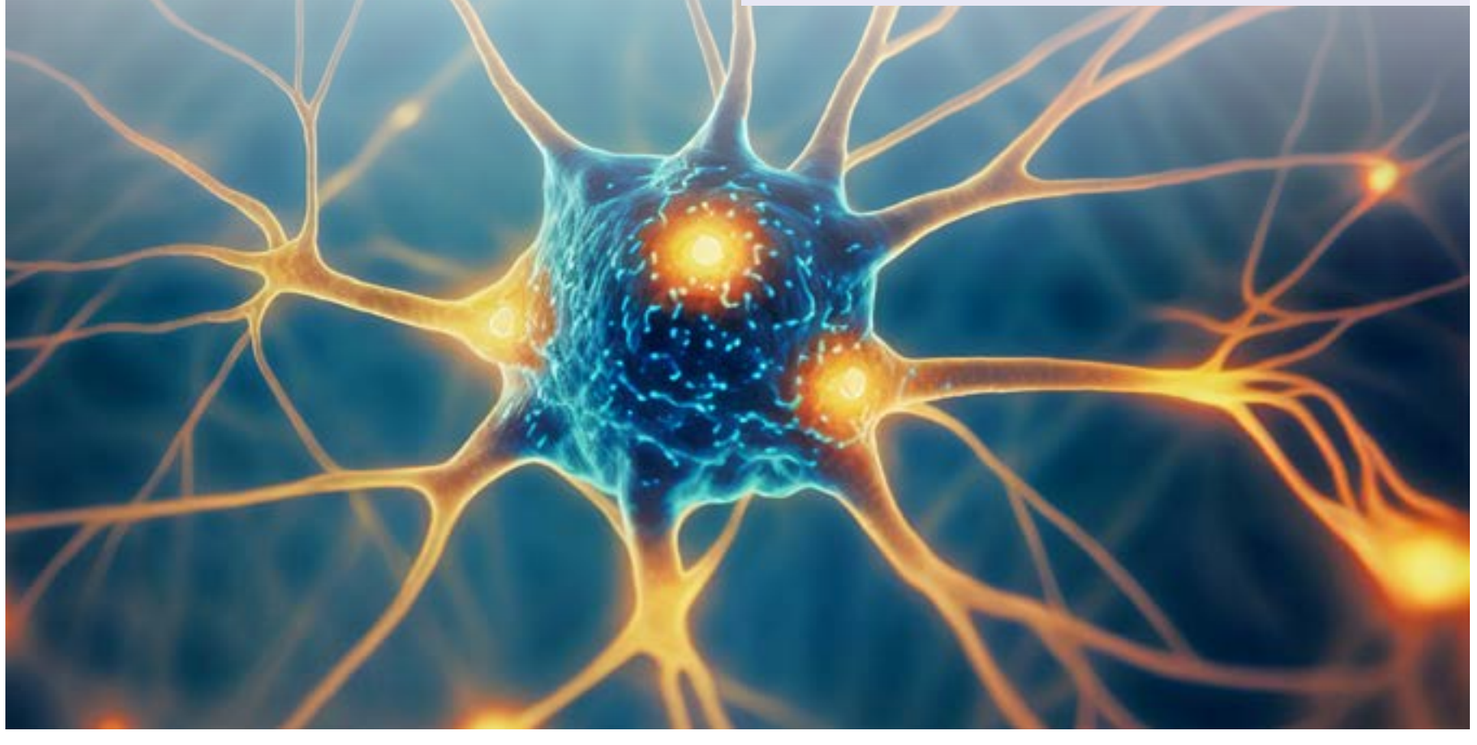
on the RAR and RXR receptors. They also tested the direct action of the retinoids on the kinase enzymes. To assess this, they used an immunoassay technique, a method based on the ability of an antibody to bind to its respective antigen, in this case, the activated ERK1 and ERK2 kinases, to identify them and to measure their levels inside the treated cells.

Finally, the researchers looked for neurite outgrowth in the SH-SY5Y nerve cancer cells by using special stains that helped to enhance the images of the cells under the microscope and allowed the measurement of the average total length of their neurites. They also calculated the number of cells in the final solutions, after their treatment with the various retinoids.

The double-acting agents

Out of the 28 agents tested, 19 demonstrated an effect on the cells by binding to the nuclear receptors but they all had different levels of effectiveness depending on their molecular structure. The two most potent agents were EC23 and GZ25. When it came to activating the cell enzymes, certain retinoids again demonstrated greater potential than others. This helped identify the unknown properties of many synthetic retinoids and also clearly demonstrated that the two actions are independent, with many of the agents acting on the nucleus but not on the enzymes and vice versa. What was more important, though, was that retinoids that were efficient at regulating both gene expression and enzyme activity (both genomic and non-genomic) directly increased the neurite length more than the other agents. These agents are termed multi-modal acting and include agents EC23, AH61, and GZ25.

These three dual-potency molecules have now been used as the basis for the design of new, even more potent drugs for the treatment and protection of nerve tissues (neuroprotection), improved nerve connections (plasticity), and nerve repair (neuroregeneration), with NVG0645 being a new lead drug that Nevrargenics is taking through the preclinical stages of development.



Personal response

How has your own experience of neurodegenerative diseases in your family motivated your work?

Like all families, the silent pandemic of neurodegeneration has directly affected my family. My uncle died of ALS when I was young; both my grandmothers suffered from undiagnosed 'senile dementia'; my father died a few years ago from Alzheimer's disease; and my wife has progressive supranuclear palsy (PSP), a rare and terminal neurodegenerative disease.

What inspired you to focus on synthetic retinoids in particular?

Retinoids are crucially important molecules in a huge number of biochemical processes that are key to proteostasis and cell health and development. Although these compounds have been looked at in the past, particularly for cancer, they have not been re-examined for treating neurodegeneration. They are clearly an immensely important class of compounds that cannot be ignored any longer, and especially given the total lack of success of other classes of drugs for treating neurodegeneration.

In your research, are there any findings that surprised you?

Everything we have done has been surprising – that's the beauty of doing blue skies and cutting-edge research. You can let the science teach you, and not second guess the results from well-designed experiments. That means everything you find out is new and exciting. The dual-acting nature of our retinoids is especially exciting because we now know that we can control numerous different biochemical processes. Yes, this is complex, but really complex diseases need really complex solutions and that includes multi-modal drugs.

How do you see these findings applied in the very near future?

We have a lead drug that is undergoing late-stage preclinical and efficacy studies; that drug looks promising. The next stage is more advanced model testing and then into the clinic, starting with ALS and/or FTD patients, checking safety and efficacy in patients.

What do you think the next natural step in your research field will be?

Complete the efficacy studies, continue to investigate the complex network of mechanisms that such compounds control, and then design and develop the next generations of drugs to tackle a number of different neurodegenerative diseases.

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Funding

EPSRC, BBSRC, MRC

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- Professor Ehmke Pohl and Dr Paul Chazot, Durham University
- Professor Susan Duty, Kings College, London, Dept of Pharmacology and Therapeutics

Bio

Andy Whiting is Professor of organic chemistry at Durham University. He has collaborated widely with biologists, especially working on the control of cellular development, which led to the design and use of synthetic retinoids to examine retinoic acid signalling pathways. Andy is founder and CEO of Nevragenics, where he is developing potential neurodegenerative disease treatments, with particular emphasis on ALS. He has also developed fluorescent imaging analogues of the synthetic retinoids being applied for cellular imaging and phototherapeutic applications through LightOx Ltd, for whom he was founder and CTO.

Further reading

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- Tomlinson, CWE, et al, (2021) Structure–functional relationship of cellular retinoid acid binding proteins I and II interacting with natural and synthetic ligands. *Acta Crys, D77*, 164–175.

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