Impact Objectives

- Treat rheumatoid arthritis using the targeted delivery of carbon monoxide, relying on its anti-inflammatory properties
- Produce targeting agents with high affinity to pro-inflammatory cytokines
- Work on the development of gold nanoparticles with carbon monoxide releasing molecules, either directly bound to the surface of the nanoparticles or to an intermediate layer of bovine serum albumin.

The right release

*Treatment of rheumatoid arthritis generally consists of anti-inflammatory drugs, but here **Teresa Santos-Silva** explains why she hopes carbon monoxide will become the treatment of the future*

How exactly is carbon monoxide (CO) used to treat rheumatoid arthritis?

Rheumatoid arthritis is an incapacitating autoimmune disease that affects people of all ages, worldwide. It is a condition characterised by an overproduction of pro-inflammatory cytokines in the joint cavities, which induce joint destruction.

CO is a signalling molecule produced during the degradation of haem groups, which triggers a cascade of cytoprotective mechanisms in stress and inflammation situations. The exact way that CO elicits its anti-inflammatory effect is not yet fully understood, although some pathways are known. At low levels, CO acts as a strong anti-inflammatory and anti-apoptotic agent, preventing endothelial damage due to oxidative stress and actively promoting endothelial healing. Carbon monoxide releasing molecules (CORMs) have already been used in many animal models of disease with impressive results, treating rheumatoid arthritis and other pathologies.

Can you share a little about how your project has developed to date?

During the first year of the project, we have been dedicated to the development of targeting agents with high affinity to pro-inflammatory cytokines. We have also looked at the functionalisation of gold nanoparticles with CORMs, either directly bound to the surface of the nanoparticle or to an intermediate layer of bovine serum albumin.

One of the major challenges when working with CORMs is the detection of CO release *in vitro*. Many reports in the literature fail to mention the intense fluorescent background obtained when the popular palladium complex COP1 is used in biological media to detect CO release. From our initial experiments, we realised that albumin or simply Dulbecco's Modified Eagle Medium also trigger a fluorescence response of COP1 over time.

There has been a lot of work on CORMs; why is there still such a need for new research?

Most of the CORMs used so far do not obey the specifications and profiles required by proper drugs. Designing CORMs with a drug-like profile that deliver CO in a target-specific manner is very challenging, and requires fine tuning of the metal core and ancillary ligands. So far only one drug-like CORM has been reported, which targets the liver for the prevention of acute liver failure, namely that caused by paracetamol intoxication. Our goal is to develop new agents using a modular approach that can tackle the lack of specificity of CORMs. With this strategy, CO release is achieved in the desired tissues or organs more rapidly and with lower doses.

Are there any side effects associated with using CO in this way?

The results obtained by the scientific community in the last two decades show that even experimental CORMs are good vehicles to deliver CO for anti-inflammatory therapeutics, producing curative results devoid of any recognisable toxicity in rodent models even after one-month daily treatment. Upon administration CORMs release CO and disintegrate, generating metal containing metabolites. In the case of Mo-based CORMs, soluble polyoxometallates are formed that interact through hydrogen bonds with the surface of proteins according to the solved 3D structure of a model protein, which was crystallised in the presence of a molybdenum CORM. Ru-based CORMs react with and are transported by serum albumin without apparent adverse effects.

Can these methods be used to selectively transport other drugs to focused areas of the body?

In this project, we are focusing on rheumatoid arthritis as a proof-of-concept. We believe that the same strategy can benefit many other pathological situations where inflammation plays an important role. By combining other sets of CORMs and antibodies, other types of tissues or organs can be targeted, potentially helping to fight cancer or infection. In the future, we might find the carbon monoxide guided shuttles (COGSs) we are working on in combined therapies, as the impact of CO is extended to other pathological situations.

Treating arthritis with carbon monoxide

*Carbon monoxide tends to be associated with air pollution and dangerous household leaks, but the gas is now being investigated in the **COGS** project as a viable treatment for arthritis*

Rheumatoid arthritis is a serious disease that attacks the patient's joints, and which is thought to affect around 1 per cent of the world's population. It can affect anyone, but its onset is normally at around 40-50 years of age, becoming increasingly painful as the patient gets older. Characterised by stiff and painful joints, rheumatoid arthritis is a chronic process that appears to have a number of quantifiable causations, such as alcohol and red meat consumption, as well as genetic factors. Prevention, on the other hand, is difficult to characterise, making therapeutic options vital.

Rheumatoid arthritis results from an autoimmune response that sends antibodies to attack the joint linings, known as the synovium, leading to damage and inflammation in the cartilage, tendons and ligaments. The treatment of this disease generally entails drug therapies that use analgesic and non-steroidal anti-inflammatory drugs as well as steroids and disease-modifying anti-rheumatic drugs.

TARGETED DELIVERY

Supplying a drug to its point of need is known as targeted drug delivery, and is intended to carry out two main functions. It allows for higher concentrations to be supplied to where they are needed, and it allows materials that may be detrimental to the body per se but locally advantageous to be used without risk. Typically, targeted drugs are delivered as a cloud of nanoparticles that make their way to the intended site and supply the therapeutic element as required, and without risk to the rest of the physiology.

This is the ideal situation with a condition such as arthritis, which is highly localised and requires large concentrations of relieving drugs to be effective. While there are also a range of physical therapies such as exercise routines and even surgery that can be used to alleviate the effects of arthritis, there is growing interest in the use of targeted delivery drugs. The range of drugs available are generally effective, but

> *"Our main objective is to create target specific agents that can release CO in a controlled and site-specific manner, which we called carbon monoxide guided shuttles (COGSs)"*

what appears to be one of the most potent is also highly poisonous – namely, carbon monoxide (CO). Getting CO to the area of need is difficult, and overcoming this difficulty is the basis of the COGS project, led by Dr Teresa Santos-Silva of UCIBIO, Universidade NOVA de Lisboa, Portugal.

HOPE IN A SILENT KILLER?

CO is more commonly known as an agent of air pollution. It is the 'silent killer' that can escape from fuel burning household appliances if they are improperly installed or maintained. But research has shown that CO is also an effective anti-inflammatory agent, and this has piqued researchers' interest in its possible use as a therapy for the treatment of arthritis.

CO has been shown to help inflammation by triggering cryoprotective mechanisms at a local level, meaning that if it can be introduced at the source of the inflammation, it may well be a far more effective therapy than any other currently available. The nature of CO's anti-inflammatory properties is not yet fully understood. However, some of the pathways involved, such as the promotion by CO of anti-inflammatory substances called cytokines, are known. While work is continuing in this area, Santos-Silva and her colleagues are investigating effective means of delivery.

SAFE DELIVERY

Because of its highly toxic nature, the method of actually getting the CO to the point at which it is needed without adversely affecting the rest of the body is of key interest. Crucial to the project are so-called carbon monoxide releasing molecules, or CORMs. CORMs are well understood, and as their name suggests they can be used to release controlled amounts of CO to tissue and cellular material. However, they currently lack the level of tissue specificity

Team members and external collaborators in the COGS project (left to right): Fernando Cardoso (IHMT-NOVA), Maria dos Anjos Macedo, Rita Mendes, Pedro Viana Baptista, Alexandra Fernandes (UCIBIO-NOVA), Inês Lopes (IMM-ULisboa), Teresa Santos-Silva, Maria João Romão (UCIBIO-NOVA), João Eurico Fonseca (IMM-ULisboa), Cláudia Nunes (FF-UPorto), Bruno Vidal (IMM-ULisboa), Andreia Marinho, Marino Santos, Jayaraman Muthukumaran (UCIBIO-NOVA) and Carlos Romão (ITQB-NOVA)

to make them effective for use in inflamed areas. 'This limits their use as therapeutic agents, as the higher doses needed may pose toxicity issues,' says Santos-Silva. Most synthetic CORMs are metal carbonyl complexes composed of a central transition metal with CO and other ancillary ligands. While well-structured they are not sufficiently controllable to supply doses with accuracy. 'Our main objective is to create target-specific agents that can release CO in a controlled and site-specific manner, which we called carbon monoxide guided shuttles – COGSs,' she explains.

Santos-Silva's pioneering studies into X-ray crystallography of CORMs led to the central premise behind the COGS project. With a background in organic chemistry and a strong collaborative association with Professor Carlos Romão from Portugal's Instituto de Tecnologia Química e Biológica, who is regarded as an expert in CORMs, she is in a strong position to push the project forward. Key to the initiative is the development of a suitable carrier for the CO. Currently, the researchers are considering the use of gold nanoparticles, which have been previously used in many biomedical and biotechnological applications.

As well as Santos-Silva herself and her group, the team that she has gathered is well placed to undertake this investigation. It incorporates four groups, and includes researchers with interests spanning everything from technology to medicine. Dr Pedro Viana Baptista and his team, also at the Universidade NOVA de Lisboa, bring with them a long track record on the functionalisation of gold nanoparticles for medicinal purposes. Another NOVA researcher, Dr Fernando Cardoso, is producing antibodies specific for the inflammation site in rheumatoid arthritis using phage display techniques. Also at NOVA, Professor Maria dos Anjos Macedo is an expert in structural biology and is responsible for the NMR structural characterisation of the targeting moieties. At iMM Lisboa, meanwhile, Dr João Eurico Fonseca is leading the more hands-on side of the project. As a clinical doctor, he has experience using CORMs to treat rheumatoid arthritis. Besides these team leaders, the project is receiving expert guidance from Romão, and from well-known crystallographer Professor Maria João Romão, who was also involved in the structural characterisation of protein-CORMs complexes.

NANOPARTICLE SOLUTION

It has already been determined that the use of antibodies conjugated to such metallic particles introduces a much higher level of targeting ability compared to that obtained with other small molecules, making the remedy more likely to be delivered where it is needed. While the entire development of the therapy incorporates many aspects, Santos-Silva's team is looking to characterise the conjugates – molecular species featuring two or more distinct components that result in a single reaction – which form the gold-CO complex.

The team hopes to employ a modular approach to the problem, and construct the COGSs from the ground up. The initial investigation has focused on how the CORM interacts with biological proteins, the process of binding to side chains, and how the ancillary ligands are replaced by water, thereby releasing CO as required. This versatility means that CORMs can be either very simple or highly complex in design, depending upon the delivery required. The last few years have shown that CO can strongly behave as a regulatory molecule at pathophysiological concentrations, providing therapeutic effects in many cellular and biological processes. However, translating this from theoretical and experimental work into real world solutions still requires work. The main thrust of the investigation is now focused on establishing the CO release rate when in a biological environment.

Rheumatoid arthritis is a debilitating illness that can restrict a person's movements at the time of life when exercise and mobility is becoming ever-more important. Relieving inflamed and swollen joints will promote activity, leading to improved overall fitness, and potentially increasing lifespan. While there is still fundamental work to do, the initial results from the COGS project are promising and the multi-structured team have a practical result firmly in their sights, which will be a huge relief to millions of arthritis suffers.

Project Insights

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Professor Teresa Santos-Silva completed her PhD in the Department of Chemistry at Universidade NOVA de Lisboa, Portugal, and has worked there since as a postdoctoral and then research assistant professor. She specialises in structural biology, using X-ray protein crystallography and small angle X-ray scattering to characterise protein-ligand interactions for structure-based drug design studies. She has focused on the crystallisation and structure determination of metalloproteins, and the 3D structure of CORM-protein-adducts.