Anti ANCA Patches

Overview

ANCA Associated Vasculitis (AAV) is a type of vasculitis which affects small blood vessels around the body, causing inflammation within blood vessels. If left untreated this could lead to: further vessel damage, organ failure (due to insufficient blood supply), and even death (1). AAV specifically stems from the autoantibodies called ANCA. The main 3 types include: Granulomatosis with Polyangiitis (GPA), Microscopic Polyangiitis (MPA) and Eosinophilic Granulomatosis with Polyangiitis (1). The combined incidence rate in the USA (1996-2015) is 33.0 per million and 1.5 per million in the UK as of 1980 and these numbers are set to increase in the coming years, as they have already increased within the last 40 years. The 5-10 year survival rates of a patient with vasculitis are between 46-85% demonstrating that treatment can be inconsistent among patients (4), therefore a longer term consistent treatment is fundamental to controlling the disease. Additionally, research has shown that the ratio of Males:Females is much higher in PR3-AAV than MPO-AAV, which was supported further by a Japanese study into GPA specifically. Moreover the the average median age of all AAV conditions is 63.5 years. As the map shows, the highest density of MPO-AAV is located in Asian populations such as China and Japan whereas the majority of cases of PR3-AAV are in the Western hemisphere and the Middle East, this could hint at possible environmental or genetic factors that make people predisposed to have AAV (27)

The Science

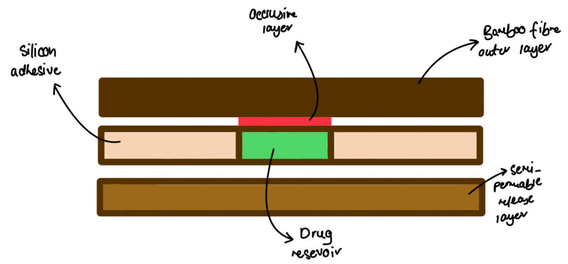
There are two types of ANCA autoantibodies, (PR3)ANCA and (MPO)ANCA which are able to bind to the enzymes, Proteinase-3 (PR3) and myeloperoxidase (MPO) respectively. The enzymes are usually intracellular, however during the degranulation of neutrophils they can be released (13). The distribution of these two molecules varies between types of AAV. In patients with GPA 80% of the ANCA present is of the PR3 type with 15% being MPO, whereas patients with MPA have 20% PR3 and 70% MPO distribution (4). The binding of ANCA can cause reactive oxygen species (ROS) and lytic enzymes to be secreted, as well as initiating neutrophils to migrate through endothelial walls (14). These factors cause damage to the endothelial wall causing inflammation to occur. Recent extensive research has proposed the idea of synthesising nanozymes which are able to bind to 2 target molecules which could be used in our design to target both types of ANCA (8)

Directions for use:

1.) Thoroughly wash and dry the area of application
2.) Peel plastic off of the sticky side of patch, taking care to keep this side face up whenever possible
3.) Carefully apply the patch to the side of a stretched but straight neck, to minimise air bubbles and maximise skin contact
4.) Remove and repeat the process daily, alternating the side of the neck used as to not irritate the skin (15)

IMPORTANT: Even though the drug is contained only in the centre of the patch to ensure the full dose is administered, touching the sticky side whilst applying the patch may result in the weakening of the adhesive making it start to fray before the end of it's 24-hour lifespan. The consequence of this may be a decrease the skin contact of the patch.

Cross-Section Of Patch



Drug Reservoir
- Even if the edge of the underside is touched during the application, there is a low risk of a loss of dosage as the drug is only stored in the centre
- The reservoir is as thin as possible to make it more discreet
- Thin drug layer has a higher surface area to accommodate for the correct volume of drug - higher surface area allows for a greater rate of diffusion

Semi-permeable membrane and release
- Covers the whole bottom of the patch to ensure maximum contact with the skin for diffusion
- Allows controlled release of the drug - ensures required lifespan
- Maintains the correct concentration gradient over the 24-hour period to give the optimum rate of release

Occlusive layer
- Occlusive (non-permeable) polymer layer on the top and sides of the drug reservoir to only allow diffusion down towards the skin

Silicone-based adhesive
- Located on the edge of the patch, surrounding the reservoir
- Less likely to get the drug stuck in it if it's out of the way of the path of diffusion
- More appropriate than acrylic (16) since it is not too strong so it is less likely to damage or irritate skin (18) - able to leave on for required 24 hours.
- Comes off with minimal residue (15) - although it is still important to clean the area of accumulated skin and pathogens
- Forms a waterproof layer on the skin (18), which helps to reduce the effect of sweat and other moisture on the strength of the adhesive

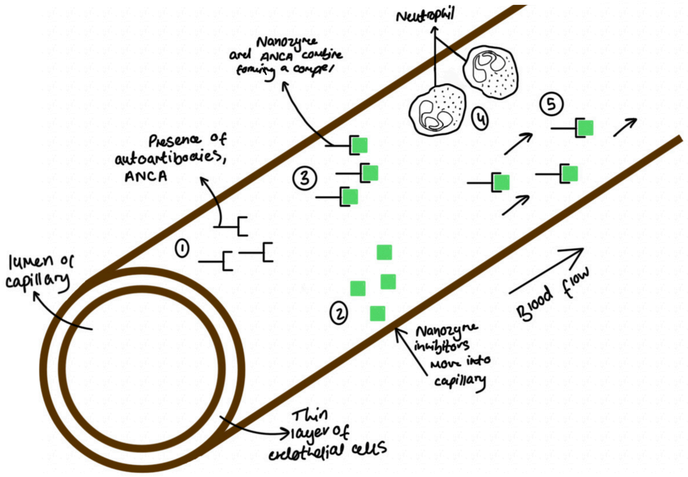
Our Solution

In order to stop the neutrophils from secreting harmful substances we have taken an approach which utilises nanozyme (6) technology to mimic the structure of PR3 and MPO to intercept the ANCA so they cannot bind to neutrophils. We chose an approach that was specific to the ANCA as to not disrupt the activities of neutrophils which play a key role in the immune system.

The Process

1) There are ANCA antibodies present in the blood secreted from lymphocytes.
2) Inhibitors which have moved through the skin diffuse through pores in the capillary and enter the blood stream.
3) The ANCA inhibitors are specific and complementary to the ANCA antibodies so they bind to them, forming a complex. The binding of the antibody and ANCA causes the fluorescent group to be activated. (26)
4) Presence of the inhibitors greatly decreases the probability of the ANCA antibodies binding to neutrophils, therefore less like to trigger a response of the neutrophils.
5) antibody-inhibitor complexes then move through the blood to be excreted where it can be tested (11)

Demonstrating Process Of Inhibition



Nanozymes

A nanozyme is a nanoparticle which mimics the specificity of a biological enzyme (6). Nanozymes have great potential in medicine due to a range of factors which make them advantageous compared to natural enzymes. Examples include: Higher efficiency and stability (10), lower production costs and can increase the efficiency of ELISA tests since they do not need to be purified and prepared. Interestingly, platinum-based nanozymes have already a found a use as anti-inflammatory treatment, as they resist against ROS. (7)

Testing Element

Whilst treating the disease is of most importance, it is also vital to make sure that the treatment is actually working effectively and that the treatment plan and diagnosis is correct. Treatment being inconsistent was a flaw we identified in current patients, therefore, our design integrates testing into the treatment. This is done by collecting a urine sample and analysing the concentration of fluorescent antibody-inhibitor complexes, to determine if treatment has been effective. For example if a patient has just started treatment and has been tested, and shows no fluorescence in their urine, this could indicate that antibody-inhibitor complexes have not formed, so this form of treatment is not appropriate. This is a pivotal part of the design because it tells us if the treatment is effective in real time, this is advantageous because it means time is not wasted and the optimum treatment can be evaluated at a quicker rate for the patient, to ensure quicker recovery and decrease the chance of relapsing.

Trials

The consideration for the safety and efficacy of the drug is a very important step in the development of the product. The are a number of trial stages involved:

1) Pre-clinical trials involve testing on cultures of cells in a laboratory. If the drug harms body cells, it will not be suitable for further development.

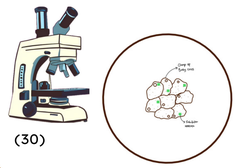
2) Animal trials are the next stage, where the safety and efficacy of the drug on a whole organism can be tested. A range of dosages are used to see if there are serious side effects as the dosage increases.

3) Next, we need to test the drug on healthy people in order to determine if the drug has any side effects or adverse effects on specific factors e.g. gender, age, weight. The patients who have received the treatment are then observed in case any side-effects emerge. This acts as a control variable

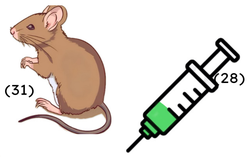
4) Finally, the drug can be given to people affected by AAV to optimise the dose, as well as determining any additional side-effects. Fluorescence in the urine is measured to observe the point where an increase in dosage no longer has any medical impact on the patient - allowing the correct dose to be deduced.

Prototype Patches

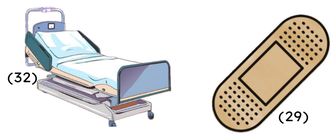
1) Pre-clinical



2) Animal trial



3) Healthy Patients → 4) Patients with AAV



Rejection

While nanozymes are typically made up of inorganic material we wanted to ensure that the body does reject the nanozymes making them redundant. To mitigate chance of rejection we are going to envelope our nanozymes in lipid-based molecules (similar to micelles), which make them ideal for drug delivery since they are, non-toxic, non-immunogenic and can release contents at a desired location. This allows us to overcome the boundary of rejection for more efficient delivery and treatment. (33)

Feasibility

Location - With the drug being administered to the common carotid artery, it will make it to many areas affected by AAV (sinuses, nose and throat). This area is not too hairy so the patch can adhere better to the skin and will be less painful to remove.

Patch Design - Having the drug separate from the surrounding adhesive (17) makes it ideal for application in the case of older patients (e.g. if they are not as good with fine movements or if a carer is applying the patch for them).

Ethics - A vital part of the testing process is performed on live animals, while the conditions will be controlled to minimise adverse effects and death, we recognise that this part of the process raises ethical issues which we aim to overcome.

Sterilisation

- Sterilisation is vital since the patch is in constant contact with the body so it has to be clean reducing the chance of infection.
- The process can make up over half of total costs (19) so batch sterilisation is more favourable - lower price per part
- Electron beam sterilisation (21) fires radiation at the component - must be low level to avoid damage due to ionisation
- Chemical methods are not favourable as they may affect formulations in the patch or introduce an irritant to the skin
- ISO cleanrooms (20) (space where low particle concentrations are used to keep processes sterile) can be used to test the manufacturing of the product and the effectiveness of different sterilisation methods

Inclusivity

Adhesive technologies such as plasters and contraceptive patches are infamous for being available in exclusively Caucasian shades (22). Concerns are often raised that use of these products acts as a reminder to ethnic groups of a prejudice that remains in today's society (23). Whilst certain supermarkets have branched out into more inclusive options, the pharmaceutical industry still has a long way to go on the matter. It is our belief that products, such as Anti ANCA Patches, will help accelerate change in this field by making it a key feature of our design. An increasing number of studies have shown the strong link between mental and physical health (24), and so it is vital that when considering any medical treatment, to also consider how it will affect mental health, since it may effect the speed of the treatment. Inclusivity is vital, and small adaptations in every day life ensures that everyone feels represented as countries embrace their multicultural population.

Social Acceptability

Currently, nanozymes are still undergoing research and we do not fully understand the interactions that they could have with other molecules in the body. This is something which we need to test during pre-clinical trials. Also the idea of synthetic enzymes mimicking biological enzymes may cause anxiety for some. We also plan to have education strategies in place with key representatives of all key groups to increase acceptance.

Team Members:

Theo Mikkelsen: Biology, Chemistry, Maths
Toby Watts: Physics, Chemistry, Maths

Team Roles

Theo - images/design, nanozyme research, feasibility
Toby - Patch design/research, inclusivity research, feasibility

UNDAUNTED

VASCULITIS UK

IMPERIAL

References