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Title: Sheep as a pre-clinical model for human gene therapy
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Introduction: Genes are the instructions for building living bodies. However, a gene can become 'mutated' and fail to provide the correct instructions. 'Batten disease' is an example, mutations that cause a rapid loss of function of the eyes and brain that begins at birth and ends in blindness and early death. My supervisor and I are studying Batten disease using sheep, as it occurs naturally in sheep and sheep are also very like humans in terms of brain size and shape, allowing findings to be easily applied to humans.

A proposed treatment for Batten disease is 'gene therapy': inserting a correct copy of the mutated gene into affected cells. Viruses are used, as previous researchers have established safe methods of replacing harmful viral genes with a useful gene of our choice, which the virus then inserts into the cells we want to treat. However, getting the virus into the skull-surrounded brain is a difficult problem. Two routes have been tried: 'Intracerebroventricular' (ICV) and 'Intraparenchymal' (IP). The ICV route uses large fluid-filled spaces at the centre of the brain that connect to a fluid that flows around the entire brain and spinal cord. The IP route simply directs virus directly into brain tissue. Each route has pros and cons, mostly centred around risk of damage. The IP route requires hundreds of injections into the large human brain to ensure enough viral spread, with each injection risking fatal inflammation or infection. In contrast, the ICV route only needs one injection, but it is a particularly deep and therefore risky injection. I compared these to the 'intracisternal' (IC) route. This uses the cisterna magna, a small pocket of fluid at the back of the brain that connects to the same fluid that the ICV route connects to. It was hoped that the IC route, being easy to access but also connected to a convenient flow throughout the brain, would be both easier and safer than the IP and ICV routes. Therefore:

Aim: To assess the spread of gene-carrying (AAV9) viruses through the healthy sheep brain after injection into the cisterna magna, and to compare the distribution of the gene and its intensity to previously documented intracerebroventricular and intraparenchymal routes.

Impact: Batten disease is rare, but is very similar to a host of fatal genetic diseases caused by a similar mutation. Individually, each disease is very rare, but the sum total is close to common. Through this studentship project, future researchers aiming to treat these diseases with gene therapy will be able to divert their time to the most effective routes.

Method: Disease-specific genes can be expensive to produce and hard to detect, so we used AAV9 viruses loaded with the cheap and easily detected gene for Green Fluorescent Protein (GFP). These viruses were injected into the cisterna magna of two healthy sheep. After four weeks, the sheep were euthanised and their brains (and small sections of some other organs) were collected. These were fixed in formalin, stored in cryoprotective fluid and then cut into 0.05mm thick sections. Every 40th section was washed and immunostained such that any GFP turned deep brown. This staining allowed the number of the cells expressing the GFP gene to be recorded, as a sheep brain cell will not normally produce GFP. GFP, therefore, can only be present if the AAV9 virus had successfully inserted its GFP gene into the cell.

Specific regions in the brain were defined and viewed through a light microscope. Using a predetermined magnification of 10x, I counted the number of stained cells in 20 fields of view (FOV)

for each region and then obtained an average number of stained cells for each region. This differentiation by region, combined with taking every 40th section, allowed the GFP uptake through the entirety of the brain to be assessed. This was then compared to the work of my supervisor, who had done the above for the ICV and IP routes.

Results: The IC route resulted in low or no intensity of GFP staining. This made it much less effective than both the ICV and IP routes, as the ICV route had moderate intensity across the brain while the IP route had high intensity around the injection sites and low intensity in the rest of the brain.

Across all brain regions for both sheep, the average number of GFP-expressing (stained) cells was 1.4 cells per 10x FOV. Many regions had the minimum average of 0, while the frontal cortex had the maximum average of 6.5.

In comparison, overall averages were 58 GFP-expressing cells/FOV for the IP route (maximum of 264 and minimum of 1) and 45 cells for the ICV route (maximum of 95 and minimum of 9).

Conclusion: Injection of GFP-carrying AAV9 virus into the cisterna magna of healthy sheep did not result in clinically useful GFP uptake. It is highly unlikely that this experiment failed due to an ineffective vector, as testing the IC route is not entirely new; my supervisor tried it previously, and also failed to find significant GFP uptake. Since she used a lower concentration of a less effective form of virus, this second trial which formed my studentship and tested higher volumes of a more efficient AAV9 virus was considered necessary. However, since it gave the same results, it is safe to say that treatment of genetic diseases of the brain using gene therapy given by this delivery route are unlikely to work.

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