ERN Support and Training programme- WP17- EJP RD

Report Workshop "The Blood-Brain Barrier: current research and novel therapeutic crossing approaches"

Date/Venue/Format

The workshop dedicated to the Blood Brain Barrier (BBB) took place in the Aula Magna of the Department of Paediatrics of the University of Padua in Padua, Italy.

Date: 8th - 9th June 2023.

The event was conceived from the beginning as an on-site workshop with no remote participation via streaming tools. However, due to the unforeseen personal issues of two invited speakers, one presentation was held remotely, with one speaker connecting from another location.

Numbers (participants, speakers, ERNs represented, patient representatives)

8 speakers and 10 participants

Summary of the presentations and discussions

Day 1

Greg Pastores: introduction to neurodegeneration

Dr. Pastores gave started from the difference between neurodegenerative and neurodevelopmental disorders to show how the classification of neurological diseases is based on primary clinical features, anatomic distribution, and principal molecular abnormalities.

He then explained how the neuronal vulnerability is characterised by the inability to replicate, accumulation of age-associated damage to cell components, high energy requirements, and complex architecture. In this context, autophagy in the cells of the central nervous system (CNS) represents the main mechanisms to prevent accumulation of cellular proteins and damaged organelles. The main hallmark of neurodegenerative disease is the pathological protein aggregation (which acquire neurotoxic properties). Lysosomal dysfunction has a major role in neurological disorders (neurodegenerative storage diseases). There are significant overlaps with other hallmarks of aging, which is a risk factor for neurodegenerative disease.

Various genes have been identified with a role in neurological diseases by initiating or propagating the disease process, but finding a gene related to a certain condition is not enough because of varying penetrance.

Starting from Prof. Scarpa observation that there are phenotype variations of the same disease among syblings, Dr. Pastores highlights that there are other nongenetic factors that influence the expression of the disease. Genetic sequencing does not provide all the answers, although it can show that some phenotypes are the result of many mutations.

Angeles Garcìa-Cazorla: introduction to neurometabolism

Dr. Garcìa-Cazorla introduces the neurometabolic disease: a large family of diseases in which the involvement of brain in metabolism is important. New categories of diseases have appeared recently, also due to a new classification. The simplified classification of neurometabolic diseases is based on small molecules, energy defects and complex molecules.

Overview of diseases based on classification:

• Small molecules: intoxication diseases characterised by neuropsychiatric problems as baby grows; deficiency diseases with a spectrum of severity, some are treatable (patients starting treatment early, within 1st year, have a much better development vs. those who start later).

• Complex molecules: many new treatments and biomarkers are now available, rapidly increasing knowledge in basic science. Deficiency of phospholipids is relevant, as is cell trafficking.

• Energetic disorders: new diseases not related to respiratory chain but to transport of organelles. Brain metabolism is affected in all kinds of rare genetic neurological disorders, not only metabolic diseases. As an example, Dr. Garcìa-Cazorla shows an overview of patients with specific diseases (photos and videos) and the effect of therapy on their symptoms.

Elvira De Leonibus: targeting early stages in neurodegeneration by boosting

autophagy

Dr. De Leonibus highlights the fact that targeting early stages is crucial in neurodegeneration to delay neuronal dysfunction and she introduces TFEB, a master gene that stimulates autophagy, as a starting point to answer the question on how to stimulate autophagy safely and with control to treat neurodegeneration. Dr. De Leonibus shows how her group managed to safely stimulate autophagy through spermidine which, by stimulating TFEB, rescued memory impairment and cleared protein aggregates in the brain of mice.

The same molecule was tested in mucopolysaccharidosis type IIIA, where it

increased the expression of TFEB in the brain of mice, favoured secondary storage clearance, and improved behaviour. Based on these results, spermidine represents a potential good drug candidate to delay dementia.

<u>Reinhard Gabathuler: structure and function of the BBB</u>

Prof. Gabathuler starts by highlighting the positive aspects of the BBB, which protects neurons and synapses in a tight environment and regulates brain homeostasis. However, this also means that a lot of therapeutics cannot reach the brain, so in case of CNS disease, it is difficult to reach the therapeutic concentration without systemic toxicity.

After an overview of the routes to cross the brain endothelium, Prof. Gabathuler presents some approaches to cross the BBB:

• Invasive techniques: not patient friendly and with diffusion in the brain parenchyma;

• Pharmacological approach: modify drugs chemically to achieve passive diffusion;

• Physiological routes: diffusion to target 10-25 μm.

Multiple approaches are now available to deliver drugs to the CNS:

• Use of transporters expressed at the luminal and basolateral side of BBB: probably more applicable to small drugs;

• Use of absorptive endocytosis and transcytosis: high absorption to plasma

proteins and other organs;

• Use of receptor mediated transcytosis: specific, potential competition with endogenous ligands, need to be released in the brain parenchyma.

Prof. Gabathuler shows how the BBB is altered in lysosomal storage diseases (LSD) and concludes sowing an in vitro BBB model developed by Cellial Inc. and a perfusion method to study the BBB and the brain uptake of molecules.

During the discussion that follows, participants highlight the fact that although the approaches available are numerous, the results are still lacking and only one approach is currently in clinical trial for brain cancer.

Following Dr. Fleming's question on the possibility of using microvolts to cross the BBB, Prof. Gabathuler answers that this method has not been proven to work in humans yet, as there are problems with controlling the volts and local application.

Day 2

<u>Alessandra Biffi: exploiting the potential of microglia cell replacement as a therapeutic approach for treatment of neurometabolic and neurodegenerative disease</u>

Prof. Biffi provides an example of use of human hemopoietic stem cells (HSC): genetically modified microglia cells can restore the functional microglia, modulate/reduce tissue damage and neuroinflammation, and induce effective secretion of therapeutic cells in the CNS. In the case of LSD, these cells durable delivery of therapeutics across the BBB. Gene transfer enhances the therapeutic efficacy of cell transplants: in a phase ½ trial in early-onset metachromatic leukodystrophy, lentiviral hemopoietic stem-cell were used for gene therapy, showing normalization of cognitive development. An additional application is in mucopolysaccharidosis (MPS) I and MPS II. This approach works when the treatment is given early in the disease. A novel delivery route for HSC is based on the fact that transplant-delivered cells in the brain are the progeny of a fraction of the transplanted HSC engrafted in the CNS short-term after infusion, enhancing locally to generate a microglia-like progeny.

Intra-CNS delivery of HSC results in successful, rapid, and exclusive engraftment of transplant-delivered cells in the CNS and timely generation of alocal microglia progeny. It also provides a qualitative advantage. This knowledge in LSD is relevant for adult onset neurodegenerative diseases; in particular, the progranulin defects in fronto-temporal dementia could be approached with autologous HSC gene therapy for GRN (the gene that codes for the protein). In animal models there was a significant mitigation of the disease phenotype, sign that the approach is valuable. A similar approach in Alzheimer's disease is to engineer the microglia/myeloid cells to express the TREM2 surface receptor. Another disease for application is the Nasu Hakola disease, characterized by microglia dysfunction; in this case a significant phenotype mitigation was seen in animal models.

Prompted by a comment by Prof. Scarpa, Prof. Biffi says that for translation of these approaches into humans there is a huge need for funding, so they are looking for sponsors to cover the costs of translations. The regulatory aspects are interesting, and discussion are ongoing with FDA and EMA. A key general point that is highlighted is that gene therapy does not only allow to have the missing enzyme crossing the BBB, but it also restores a healthy and functioning cell population of microglia.

Following Dr. Ardigò's question on how much needs to be demonstrated in terms of additional advantage, also from a regulatory perspective, Prof. Biffi replies that conditioning is essential to have a successful transplant and that demonstrating even equal efficacy with equal safety risk would be enough for regulators.

<u>Giovanni Tosi: nanomedicines for the brain</u>

Prof. Tosi starts by pointing out that there are zero therapeutic nanomedicines for the brain on the market, despite a high volume of research. There is a need to redefine the nanomedicine design and research, starting from what we know and what we do not know. In case of the BBB, which can be healthy like, almost ill (damaged) or completely destroyed, in case of severe damage do we need to target the BBB or can we target another cell, since the passage is destroyed? The same question applies for other states. So one "nanomedicine for all" does not apply.

To be used, the nanomedicine must be produced in large amounts, with GMP and high yield (simpler is better for big pharma), but this is not possible if the aim is crossing the BBB, because the surface of nanomedicines needs a complex engineering.

At Unimore a nanomedicine platform has been developed. Many years of research were needed to reach a proof-of-concept in non-diseased animals, and then in brain disease models (Huntington disease and Hurler MPS I and II).

A deep knowledge of the object to be delivered is needed to produce tailored nanomedicine. At the same time, protocols need to be identified for scale up of nanomedicines.

Dr. Ardigò asks if it would be possible to consider using multiple ligands/target on the same nanoparticle and if that would increase crossing and saturation, to which Prof. Tosi replies that using multiple ligands is possible, hitting different receptors mechanism; however, it could not be scaled-up. So another possibility would be to create two particles with one ligand/drug each and co-inject them. Since the nanoparticles can be administered daily, Prof. Scarpa asks if they could they be used also for metabolic diseases with daily injection, or if the efficiency of this method is too small to reach the necessary amount of enzyme. Prof. Tosi replies that it is feasible, but only if the enzyme is outside the brain.

Reinhard Gabathuler: new carrier technologies to cross the BBB

Prof. Gabathuler introduces various methods and approaches to cross the BBB:

• Invasive techniques: convection-enhanced diffusion, intrathecal infusion,

intraventricular infusion, intracerebral injection, implants and disruption of the

BBB.

• Pharmacological approaches: modifying drugs through medicinal chemistry

facilitating passive diffusion, formulation of drugs.

• Physiological approaches: use of transporters expressed at the luminal and

basolateral side of the BBB and use of absorptive endocytosis, charged peptides, cationic peptides, tat peptides and other nanoparticles. Among these approaches, the use of a receptor-mediated endocytosis and transcytosis needs to find an adequate receptor and ideal ligand. After showing the ideal peptide vector and receptor, Prof. Gabathuler gives a more detailed overview of the physiological approaches that can be used to deliver drugs to the CNS.

To conclude, results from the development of a monoclonal antibody delivered to the brain are shown together with the results of the delivery of a siRNA conjugate and the lysosomal enzyme I2S (IDS).

<u>Diego Ardigò: the Pluto project (defining disregarded rare diseases)</u>

Dr. Ardigò starts by highlighting that one big problem in rare diseases (RDs) is that there is no critical mass, so there is a limited to no knowledge base. Only 2.6% of RDs have an approved product and 21.3% have <10 papers published; these numbers are higher for diseases with orphan designation. Only a small population of RDs has showed an increase in the number of papers published over the last 20 years, which is believed to be the effect of Orphanet. RDs need more support according to the stage of research, clinical, development, and regulatory approval. The aim of the Pluto project is to find the characteristics of

RDs to push for specific support according to needs. The pipeline of progression in RDs is as follows: publications > clinical trials > orphan drug designation > marketing authorization. The main issues in this process include: inconsistencies, data integration and cleaning, diseases with no data, and definition of the disorders. On average, 66 years pass from the first publication on a RD to the first clinical trial.

After that everything accelerates: 5 years to orphan designation and 13 years to get to an approved drug.

<u>Ronan Fleming: computational models to study the brain</u>

Dr. Fleming provides an overview on the systems science approach, which includes the reconstruction of data applied to the reconstruction of biochemical networks, modelling (constraint-based), analysis, and final reconstruction of human metabolism.

After showing some examples of application, Dr. Fleming shows in detail how though this system they were able to generate a dopaminergic neuronal metabolic model for Parkinson's disease.

The presentation is concluded with a detailed description on how the whole metabolic network modelling is predictive of inherited metabolic diseases biomarkers. The rationale for application of constraint-based modelling to these diseases is also presented.