

## **POLICY PAPER**

### **COST Exploratory Workshop Pharmacology and Toxicology of the Blood-Brain Barrier: State of the Art, Needs for Future Research and Expected Benefits for the EU Brussels, 11 and 12 February 2010**

#### **Introduction**

Improved health care in the developed world, including Europe, has had a major impact on life expectancy over the last few decades. The size of the aged population in Europe has already been increasing and it is estimated that the number of people over 80 years will almost triple by 2051, to approximately 50 million. The most dramatic change will occur soon, as the post-World War II baby boomers move into old age. Furthermore, the continuing low levels of fertility will contribute substantially to the increasing proportion of aged persons in the population. It is estimated that the number of persons aged over 65 relative to those aged 15-64 will double over the next four decades.

Whilst European citizens are living longer, the burden of age-related diseases is also increasing, primarily for neurodegenerative and other central nervous system (CNS) disorders. Indeed, the World Health Organization (WHO) has calculated that by 2040, neurodegenerative disorders will be the second leading cause of death worldwide. There is also an increasing impact of brain diseases in those under 65 years. Whilst the spectrum of conditions is somewhat different than in the elderly, in both groups such brain disease-related morbidity is placing increasing demands on health care systems, resources and society more generally. The total current cost of treating mental disorders in Europe is estimated to lie between 390 and 700 billion Euros, with a societal cost of 3-4% of the gross national product.

#### **Blood-Brain Barrier: State of the Art**

There is a growing need for CNS-active therapies, requiring the development of medications that are able to reach and penetrate the desired regions of the brain. This last has proven to be problematical, as the brain is protected very effectively against exogenous substances. Its major line of defence is the blood-brain barrier (BBB). This barrier is a dynamic interface between the body and the brain, which is actively engaged in the regulatory functions of the CNS and helps in maintaining a healthy steady state. The BBB controls access to the brain of essential nutrients, vitamins and ions, as well as some proteins and peptides, and removes the products of metabolism in the brain, for example neurotransmitter metabolites. It also prevents entry of potentially harmful substances into the brain.

These functions of the BBB are a consequence of its anatomy and biochemistry, which provide the necessary structural and functional features to enable it to isolate the brain from the systemic circulation. The BBB comprises the endothelial cells of small blood vessels. These cells are underlined by a continuous basement membrane and surrounded by accessory cells – astrocytes and pericytes. Adjacent endothelial cell membranes are sealed by tight junctions, which limit permeation of all but lipid-soluble small molecules into the brain.



A range of membranal transporter proteins enable passage of those substances required by the CNS, such as glucose, essential amino acids, and neurotransmitter precursors. There are also transporters and some metabolic enzymes which function in the opposite direction, thereby preventing access to the brain of potentially toxic substances, including metabolites, which might otherwise be able to diffuse into the brain. Unfortunately, the BBB can also prevent effective drugs from entering the brain. The number of these drugs is larger than once thought.

Whilst much of the focus on the BBB has been on its role in influencing the distribution of drugs to the brain, there is increasing awareness of its potential importance in toxicology and in risk assessment of environmental stressors. Chemical substances including man-made industrial chemicals, pesticides and biocides appear to be handled by the BBB similarly to drugs. The understanding of the role of the BBB in the pathology of CNS diseases is slowly increasing. It is becoming widely accepted that inflammation, usually associated with dysfunction of metal ion homeostasis (Fe, Cu, Zn) with concomitant oxidative stress, is a key factor in a large number of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, Friedreich's ataxia, and others. Alzheimer's disease and Parkinson's disease and Friedreich's ataxia are characterised by increased levels of brain iron. Wilson's disease is caused by a genetic abnormality leading to copper overload, and copper has also been implicated in Creutzfeldt-Jacob (prion) disease. A number of epidemiological studies have demonstrated that exposure to lead induces intellectual deficiencies in children, resulting in learning disabilities and emotional/social behaviour deficits. In adults, loss of memory function has been noted and evidence for shrinking of brain volume was demonstrated. Other pollutants, such as organophosphate insecticides, are suspected of causing neurodegenerative diseases such as Parkinson's disease. The importance of active transporters and local metabolism in the BBB in causing the harmful effects of such substances remains unknown. The routes by which systemic inflammation communicates with the brain include both neural and humoral signalling, but a key feature is signalling at and across the intact BBB. Signalling of systemic inflammatory events across the BBB thus offers a potentially important target for modulation of disease progression in those with ongoing neuroinflammatory and neurodegenerative diseases.

Stressors may critically influence the development and function of the BBB in early life stages. A delayed maturation of the BBB may expose the neonatal brain for a longer period of time to toxic chemicals which are present in the human environment (e.g., lead). In later life stages, exposure to environmental stressors may have an impact on the integrity of the BBB. For example, changes in the BBB have been observed in mouse brains exposed to radiofrequency fields. There are no firm data on the integrity of the BBB in late life stages and in the aged population. However, since oxygen free radicals promote ageing and cause malfunction of human organs, it is possible that oxidative stress alters function of the BBB and thus contributes to the pathology of CNS disorders.

### **Tools and models in BBB research**

Studies of the physiological and pathological functions of the BBB are in need of good models and methodology to improve our possibilities to treat diseases that today have no cure. Studies with cultured cell models have contributed substantially to the knowledge of physiological processes in the BBB and of BBB-mediated disease. Animal models of diseases are also being utilized. Drug transport to the brain can be studied and new concepts are being developed. In spite of this we have a long way to go before diseases of



the CNS can be fully understood and cured. Translational aspects from animals to humans are of great importance but remain problematical.

Today we still know very little about how inflammatory components of systemic or CNS diseases influence drug transport into the brain, and how peptides and proteins are taken across the BBB. Drug delivery systems like nanoparticles and liposomes may work, but their impact, quantitative contributions and tolerability are not known.

### **Current status of BBB-related research in Europe**

There are several groups in Europe dedicated to different aspects of BBB research, including the development of BBB cell culture models, which started very early in Europe, and the role of the BBB in the pathology of CNS diseases. Japan and the USA also have good research in this area. The European groups are funded mainly by non-EU granting sources. Only two research projects are currently funded under European Framework Program 7 (FP7), dealing with stroke and with brain inflammation, and in particular with multiple sclerosis. In addition, there are three neuroscience networks funded by the European Cooperation in Science and Technology (COST) project within the Biomedicine and Molecular Biosciences Domain (BMBS): Action B30 – Neural Regeneration and Plasticity (NEREPLAS), Action BM0601 – Advanced Methods for the Estimation of Human Brain Activity and Connectivity (NEUROMATH), and Action BM0603 – Inflammation in Brain Disease (NEURINFNET). Although these groups are interested in the effects of drugs on disease, their focus is on the disease rather than on the mechanisms which govern the local availability of drugs used to treat the disease.

No special funding is currently provided by the EU to investigate the BBB as an important biological entity which plays a determinant role in the development of CNS-active drugs, considering that the therapeutic success depends largely on the delivery of the drug molecule to the site of action in the brain. In the USA there is awareness to deal with this topic, as exemplified by the statement paper 'Aging and the Environment: A Research Framework' (<http://ehp.niehs.nih.gov/members/2005/7569/7569.html>) and by an intramural research group within the National Institute of Environmental Health Sciences dealing with the topic.

### **Future BBB research needs in the EU – a research roadmap**

An Exploratory Workshop on the Pharmacology and Toxicology of the BBB was held on 11-12 February 2010 in Brussels, under the auspices of COST, which brought together many specialists in the area. The primary purpose of the workshop was to provide an overview of the current status of the research performed in Europe in the BBB area, with the additional aim to propose measures/strategies for the stimulation of collaborative BBB research. The workshop was attended by 47 scientists coming from 19 European countries and one scientist from the USA. Researchers within physiological and pathological fields met researchers in the area of drug development. Both basic and applied aspects of BBB research were discussed, covering broad fields of advanced science, technology and industry. The discussions among researchers with similar interests in the BBB, but different research questions and tools, were very fruitful. A broad consensus was reached regarding the needs for future research in this area in Europe, and the following open questions/challenges were identified:

- 1) Defining the basic mechanisms that drive transport of drugs and environmental pollutants across the BBB.



- 2) Identifying extracellular and intracellular signals that modulate the expression and activity of influx and efflux transporters for drugs and environmental pollutants.
- 3) Determining how diseases and environmental stressors change the homeostasis at the BBB.
- 4) Developing strategies to improve CNS pharmacotherapy by altering BBB transport function and/or using drug carriers which can enter the brain (e.g. nanoparticles).
- 5) Developing predictive tools, which is a task of utmost importance. There is a need for combined in vitro models and in vivo work. PET and other imaging methods should be utilized and compared with animal and cell models to evaluate their predictivity.

Amongst the main avenues of future research that should be addressed in the EU are the manipulation of signalling through the BBB to improve drug delivery, and determining the extent to which the barrier and its signalling systems can be therapeutic targets. Understanding how the BBB transport function is altered in the different life stages and in disease will be critical for designing effective CNS-acting drugs and intelligent drug delivery systems, as well as for understanding and preventing the toxic effects of environmental stressors.

The participants in the COST Exploratory Workshop stressed the importance of interdisciplinary research in this area. There is a need for chemists, physicists, biologists and clinicians to work together. Similarly, there need to be bridges from mathematical models to cell culture and animal models to studies in humans. Finally, those trying to increase CNS access of drugs for therapeutic benefit need to work with those concerned about unwanted access to the brain of toxic environmental chemicals.

The results of future research will have a major impact on the competitiveness of pharmaceutical companies, including small and medium enterprises which are heavily involved in developing innovative biomolecules in areas with a therapeutic need, in particular in the ageing population affected by severe CNS disorders such as Alzheimer's disease and Parkinson's disease.

Finally, raising public awareness towards the relevance and value of networking in research on the BBB is of high importance for assuring the welfare of the ageing European population. The aim of this paper is to encourage and support novel and dynamic pan-European networking in research on the BBB, as could be done within the COST system and complementary systems of the European Research Area.

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