The history of blood brain barrier biology essay

Science, Biology

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\n[/toc]\n \nInflammation accompanies many neurological disorders including Alzheimer's disease, multiple sclerosis and bacterial-meningitis, severe inflammation has been found to affect the integrity of the blood-brain barrier (BBB) (1-3). Mediators of the inflammation process (pro-inflammatory cytokines) including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) are secreted by activated capillary endothelial cells, macrophages, microglial cells and astrocytes (4). In inflammatory conditions, pro-inflammatory cytokines may be present in both the peripheral circulation and the brain tissue, in the brain tissue they can modulate the expression and functionality of ATP-binding cassette (ABC) transporters located at the BBB, for example permeability glycoprotein (P-gp) and breast cancer resistance protein (BCRP). However the effects pro-inflammatory cytokines (IL-1β, TNF-α) have on the functionality or expression of efflux (P-gp, BCRP) transporters remains contradictory, therefore the aim of this research is to investigate the effects of IL-1β and TNF-α on P-gp functionality. Understanding the explicit effects of cytokines on efflux transporters may be applicable in the pharmacotherapy of neuroinflammatory diseases as certain pro-inflammatory cytokines can influence efflux activity (5), impacting on the levels of therapeutic agents in the central nervous system (CNS). Elevated

concentration of drugs in the CNS may have negative implications, resulting in neurotoxicity or altered pharmacological effects, potential brain damage or fatality, however it may also have positive effects i. e. in meningitis or encephalitis, higher drug levels within the infected meninges or brain could enhance therapeutic efficacy. Conversely, reduced concentration of drugs in the CNS may result in therapeutic failure or form a paradigm for drug resistance.

1. 2 Blood Brain Barrier (BBB)

Brain capillary endothelial cells form a neurovascular unit known as the blood-brain barrier, a dynamic interface separating the blood from the interstitial fluid. This provides a primary defence mechanism against xenobiotics, limiting the permeability of hydrophilic substances or macromolecules into the CNS. Therefore, the main role of the BBB is to protect the brain from potentially harmful substances and to ensure correct neuronal function, consequently maintaining homeostasis of the CNS (6). Tight junctions (zonulae occludens) present between adjacent endothelial cells limit the entry of xenobiotics into the brain. This concept of an anatomical barrier between the blood and brain demonstrates a continuous wall preventing paracellular diffusion of solutes, it is important to note the diffusion of polar or high molecular weight substances are particularly restricted (6). The BBB is also defined as a pharmacological barrier, as endothelial cells express various enzymes and transporters, controlling the rate and extent of drugs reaching the brain parenchyma (CNS) via the transcellular route. Metabolising enzymes found in brain microvessels include: monoamine oxidase (MAO) A, MAO B, CYP1B1, CYP2U1 and many

others (7). Consequently, two main mechanisms (i) paracellular diffusion and (ii) transcellular transport (Figure 1), enable the transport of solutes through the BBB into the brain (8). Figure 1. Demonstration of the brain capillary endothelial cell which forms the blood-brain barrier, adapted from Löscher et al 2005 (6). There are 2 key mechanisms, which allow the transport of xenobiotics into the brain, paracellular diffusion and transcellular transport. It is important to note xenobiotic uptake into the CNS is limited by efflux transporters localised on the apical/luminal side of brain capillary endothelium. Brain capillary endothelial cells express various influx and efflux transporters (Figure 1), influx transporters uptake essential substrates such as glucose, amino acids, nucleosides and electrolytes, whereas efflux transporters limit the entry of substrates. Furthermore, efflux transporters remove therapeutic agents and xenobiotics (with polar or lipophilic properties) from the CNS restricting brain exposure (6, 9). Therefore, in neurological disorders e. g. Alzheimer's disease or brain tumours, delivery of therapeutic agents to the CNS will be restricted by the blood-brain barrier, blood-cerebrospinal fluid, blood-tumour barrier (only in brain tumours) and efflux transporters (10, 11). Influx and efflux transporters belong to superfamilies of proteins, one of the largest protein family to date is the ATP-binding cassette (ABC) efflux transporters. These membrane transporter proteins are localised on either the luminal/apical or basolateral side of the membrane (Figure 1) (6, 7).

1. 3 ATP-binding cassette (ABC) transporters

ATP-binding cassette transporters exist in all mammalian species, they are multidomain integral membrane proteins which translocate solutes across

cellular membranes against a concentration gradient using the energy of ATP hydrolysis (6). The family of ABC transporters is exceptionally widespread and functionally diverse, ABC transporters that have demonstrated a distinct role in the transport of clinically relevant drugs include Permeability glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP) and Multidrug resistance proteins (MRPs) 1-5. These aforementioned ABC transporters are all situated in the plasma membrane where they export various xenobiotics and metabolites out of the cell. The two most prominent ABC transporters expressed in brain capillary endothelial cells are BCRP and P-gp (12), for the purpose of this project only P-gp commonly referred to as ABCB1 (ATP-binding cassette, family B, type 1 transporter) will be discussed. The terms P-gp and ABCB1 will be used interchangeably throughout the project.

1. 3. 1 Permeability glycoprotein (P-gp)

Permeability glycoprotein, the first efflux transporter to be described at the human blood-brain barrier (1) was discovered by Juliano and Ling in 1976. The well-studied, well-characterised representative of the ABC transporters has a molecular mass of 170 kDa (6) and is found in many tissues including the brain capillary endothelium, malignant tumour cells, lung, intestine, epithelia, kidney, testis and primary placental trophoblasts (13). P-gp functions as an efflux transporter in the brain capillary endothelium restricting the access of xenobiotics into the CNS, whereas P-gp located in the kidneys is essential for drug metabolism and disposition (11). Further, Permeability glycoprotein efflux transporters found in the liver, have an overall role in drug hepatobiliary clearance (8). Two types of human P-gp exist: type 1 also referred to as MDR1 (Multidrug Resistance type 1), this is

encoded by the mdr1 gene, conferring the drug resistance phenotype and drug efflux at the BBB. (Only this type will be reviewed in this project). P-gp type 2, normally present in the canicular membrane of hepatocytes is encoded by the mdr2 gene, this functions as a phosphatidylcholine translocase (6). There are three mdr genes in rodents mdr1a, mdr1b and mdr2. Only the human mdr1, rodent mdr1a and mdr1b genes appear to selectively confer multidrug resistance. However only the mdr1a isoform is expressed in the capillaries (mdr1b is expressed in brain parenchyma), this solely contributes to the efflux of drugs from the brain back into the circulation (4, 13, 14). P-glycoprotein (Figure 2) located at the apical membrane of brain capillary endothelial cells (6) comprises two comparable halves, each containing six-membrane spanning domains followed by a cytoplasmic domain where nucleotide binding takes place (11). The first extracellular loop of P-gp is heavily N-glycosylated, however in vitro studies on P-gp demonstrate N-glycosylation is not necessary for the basic transport function of the transporter, subsequently, this may be necessary for aiding membrane insertion and providing stability to the plasma membrane (12). Figure 2. Permeability glycoprotein structure as illustrated in Schinkel et al 2003 (12). P-glycoprotein comprises two homologous transmembrane domains each containing six transmembrane segments and two nucleotide binding domains (NBD). The first extracellular loop is N-glycosylated. P-glycoprotein primarily functions to translocate P-gp substrates out of the cell to the external apical side of the endothelium, consequently substrates entering the endothelial cells from the brain are pumped into the blood (6). A vast number of P-gp substrates exist, the common denominator in

transported P-gp substrates is their amphipathic nature, which may relate to the mechanism of drug translocation. P-gp substrates are usually organic molecules, commonly possessing a size ranging from approximately 200 Da to 900 Da. Further, many substrates contain aromatic groups, but it is important to note non-linear or circular molecules can still cross the membrane. Substrates for P-gp are diverse, a number of therapeutic agents are substrates for P-gp including analgesics (e. g. morphine), anticancer drugs (e. g. vinblastine), Human Immunodeficiency Virus (HIV) protease inhibitors (e. g. ritonavir), corticosteroids (e. g. hydrocortisone), anti-epileptics (e. g. carbamazepine), antibiotics (e. g. erythromycin) and many others (12). Blockade or disruption of BBB P-glycoproteins significantly increase the brain concentration of various P-gp dependent drugs, this was demonstrated by Schinkel et al who established a 100-fold increase in the P-gp substrate ivermectin (antihelmintic) concentration in rat brain capillaries, whose mdr1a gene (gene encoding drug transporting P-gp) was disrupted (15). Certain agents known as " P-gp blockers" or " P-gp inhibitors" can inhibit the efflux transport function of P-gp substrates also resulting in an increased substrate concentration in the brain. This was demonstrated in an in vivo animal model where both brain levels of itraconazole (P-gp substrate) and antifungal efficacy were increased by the addition of the P-gp inhibitor GF120918 (16). Comparable findings were observed in an in vitro model which used an immortalised rat brain endothelial cell line (GPNT), intracellular accumulation of the substrate increased following treatment with P-gp inhibitor GF120918 (4). Other examples of P-gp inhibitors include verapamil (calcium channel blocker), quinine (anti-malarial) and cyclosporin

A (immunosuppressive agent) (6). Verapamil and cyclosporin inhibit P-gp through competitive inhibition, however the mode of action for other P-gp inhibitors is not known (12). P-glycoprotein activity and expression are also affected by several other factors. The first line of defence against xenobiotic and toxicant exposures are the " orphan" receptors, pregnane X receptor (PXR) and constitutive androstane receptor (CAR), expressed in human, rat and pig brain capillary endothelial cells. Exposure of CAR or PXR substrates (e. g. phenobarbital) may increase transport activity and expression of P-gp. Additionally, food additives can act as ligands for CAR and PXR, thus may contribute to altering P-gp activity or expression (9, 17-19). Research suggests many diseases are also associated with a modulation in P-gp expression or function, Human Immunodeficiency Virus infection (20), Alzheimer's disease (21), Parkinson's disease (22) and ageing (23) are reported to reduce P-gp efflux activity or expression, whereas epilepsy is associated with an increased function. The innate immune response is accountable for this modification in transporter function and expression, as it triggers the release of pro-inflammatory cytokines, examples include: tumour necrosis factor-α, interleukin-1β and interleukin-6 (9).

1. 4 Pro-inflammatory cytokines

The innate immune system response is activated by cell stress, trauma, disease, hypoxia and other stimuli, and is characterised by the release of pro-inflammatory cytokines, resulting in elevated levels in the microcirculation (24). Although the innate immune response is non-specific, the immunity provided is generally transient. Pro-inflammatory cytokines are released by activated capillary endothelial cells (12, 25), microglial cells,

astrocytes and innate immune cells such as dendritic cells, monocytes, basophils, eosinophils, neutrophils, natural killer (NK) cells, tissue-resident mast cells and macrophages (26). When the body recognises a foreign compound, for example Neisseria meningitidis, an opportunistic pathogen that is responsible for causing meningitis, the innate immune system is the first line of defence to help control invasion by the pathogen. Consequently, the activated innate immune cells or capillary endothelial cells release chemical mediators also known as pro-inflammatory cytokines and chemokines. The array of inflammatory mediators secreted include: TNF-α, interleukins IL-1β, IL-4, IL-6, IL-10, IL-12, IL-18 and interferon gamma (27) which play a role in generating the inflammatory response and recruiting immune cells to the sites of infection. However, the release of pro-inflammatory cytokines into the brain parenchyma may induce an alteration to the integrity of the BBB, in addition to its inflammatory and protective role against pathogens. A disruption of the blood-brain barrier may result in increased or decreased levels of drugs, xenobiotics or essential substrates e. g. amino acids in the CNS (2). Reduced drug entry into the CNS may be seen as a result of pro-inflammatory cytokines upregulating P-gp expression or increasing efflux transporter activity. Pro-inflammatory cytokines act on their receptors, which are present in endothelial cells, and induce a series of signals to increase the expression and efflux activity of P-gp. For example, TNF-α has been found to elevate MDR1 mRNA and protein expression, resulting in upregulation of transporter activity and a low accumulation of drug into the CNS (11). On the contrary, increased drug entry into the CNS may be observed firstly due to a disruption of tight junctions between

adjacent endothelial cells resulting in increased tight junctional permeability (2). This will allow xenobiotics to cross the BBB easily and accumulate in the CNS. Secondly, pro-inflammatory cytokines acting on their receptors in endothelial cells may also induce a series of signals to reduce efflux activity of P-gp. It is important to note brain capillary endothelial cells are similar to endothelial cells present throughout the body (I. e. intestine, kidney, liver, placental trophoblasts), in terms of expressing receptors for TNF-α, interleukin-1β and inflammogens e. g. Lipopolysaccharide (28).