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ON BRAIN OEDEMA

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LUND UNIVERSITY
Faculty of Medicine

2012

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Contusione cerebri aut meningitides laboranti
liquor in sanguinem infendatur.
Albumin convenit dare, at sal non adhibendus,
cum oedema cerebri augere possit.
Hoc experimentis in mure et fele ostendimus;
quod autem iam sciebat Per-Olof Grände.

To the memory of Jan Holst (1946 – 2006)

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List of papers

This thesis is based on the following original papers, referred to in the text by their Roman numerals.

- I** Jungner M, Grände P-O, Mattiasson G, and Bentzer P. (2010). Effects on brain edema of crystalloid and albumin fluid resuscitation after brain trauma and hemorrhage in the rat *Anesthesiology* 112, 1194–1203.
- II** Jungner M, Bentzer P, and Grände P-O. (2011). Intracranial pressure following resuscitation with albumin or saline in a cat model of meningitis. *Crit Care Med* 39, 135–140.
- III** Jungner M, Bentzer P, and Grände P-O. (2009). Prostacyclin reduces elevation of intracranial pressure and plasma volume loss in lipopolysaccharide-induced meningitis in the cat. *J Trauma* 67, 1345–1351.
- IV** Jungner M, Lundblad C, and Bentzer P (2012). Cortical capillary recruitment by rosuvastatin in experimental brain trauma is associated with increased NO production. *Submitted*.
- V** Jungner M, Siemund R, Venturoli D, Reinstrup P, Schalén W, Grände P-O, and Bentzer P (2012). Blood-brain barrier permeability following traumatic brain injury. *In manuscript*.

Abbreviations

ANOVA	analysis of variance
BBB	blood-brain barrier
cAMP	cyclic adenosine monophosphate
CBF	cerebral blood flow
cGMP	cyclic guanosine monophosphate
COP, POP	colloid osmotic pressure = plasma oncotic pressure
CVP	central venous pressure
eNOS	endothelial nitric oxide synthase
hct	haematocrit
ICP	intracranial pressure
LPS	lipopolysaccharide
MAP	mean arterial pressure
NO	nitric oxide
PGI ₂	prostacyclin
PS	permeability surface area product
PV	plasma volume
SD	standard deviation
TBI	traumatic brain injury

Background

Traumatic brain injury

Brain trauma is a major health problem worldwide, with an estimated 10 million people affected each year. Of those 1.5 million die, and among the survivors long-term disability is common, making the cost, apart from personal tragedy, in terms of healthcare and lost productivity a burden to society. (Hyder et al., 2007). Three age related peaks are present in most epidemiologic studies: in early childhood, in young adults, and in the elderly, with a male predominance especially in young adults. In childhood and senescence falls are the major cause of traumatic brain injury (TBI), whereas motor vehicle accidents account for 40–60 % all cases. The portion of trauma from violence varies widely with demography. (Bruns and Hauser, 2003, Steyerberg et al., 2008).

The mechanical primary insult will cause irreversible neuronal injury to some extent, and only preventive measures to reduce trauma incidence and severity can influence the magnitude of this damage. Medical care of brain trauma patients is directed at preventing secondary injuries to potentially salvageable cells. The pathophysiology involved in delayed cell death is complex, and include neurotoxicity from increased glutamate levels, reactive oxygen species, excessive inflammatory response, and mitochondrial dysfunction (Werner and Engelhard, 2007, Helmy et al., 2011, Fiskum, 2000). Attempts have been made to target specific cellular mechanisms with a multitude of agents, showing promise in animal studies, but have so far failed to improve outcome in clinical practice (Tolias and Bullock, 2004; Faden, 2001; Loane and Faden, 2010).

Increased intracranial pressure is devastating to the injured brain, as it will affect cerebral perfusion and oxygenation (Miller et al., 1977; Unterberg et al., 2004). Urgent surgery may be required to evacuate haematomas and contusions, whereas diffuse brain swelling is mainly treated pharmacologically. Circulatory chock, from extracranial bleeding or systemic inflammation, will further impair blood supply to the brain, and aggressive fluid resuscitation is often needed. Microvascular dysfunction after brain trauma involves vasoconstriction, adhesion and aggregation of leukocytes and platets and microvascular occlusion, further impeding nutrient flow (Hekmatpanah and Hekmatpanah, 1985; Lu et al., 2004; Lundblad et al., 2004; Robertson et al., 2011; Shohami et al., 1987). At present, prevention and treatment of ischaemia, hypoxia, and intracranial hypertension

remain the cornerstone of supportive care after brain trauma (Miller, 1978; Chestnut et al., 1993; Clifton et al., 2002; Cunningham et al., 2005).

Meningitis

Bacterial meningitis is a life threatening condition, and in the pre-antibiotic era mortality was 70–100%, depending on organism (Swartz, 2004). In the early 1900s, dramatic effects on survival in meningococcal meningitis were seen after the introduction of anti-serum from horses (Flexner, 1913), and further advances were made when antibiotics became widely available (Swartz, 2004). Still, with modern therapy, overall mortality in patients in Western countries is 5–10% in *Neisseria meningitidis* and *Haemophilus influenzae*, and 20–30% in *Streptococcus pneumoniae* meningitis (van de Beek et al., 2004; Swartz, 2004), and up to half of the survivors suffer various degrees of neurological sequelae (Grimwood et al., 2000). Since the introduction of vaccines against pneumococci and capsulated *Haemophilus* strains, the incidence of bacterial meningitis has declined, and further effects of preventions are anticipated (Thigpen et al., 2011). Adjuvant therapy with corticosteroids has also proved beneficial on neurological outcome and survival (McIntyre et al., 1997; de Gans and van de Beek, 2002). In developing countries mortality rates are around 50%, depending on age and pathogen (Koedel et al., 2002). Improvements in health care and broad vaccination programs may affect outcome and incidence in the near future (WHO, 2012).

Bacterial cell-wall components are important triggers of the immune system, and the strong inflammatory response to pathogen invasion is responsible for many of the pathophysiological perturbations seen in bacterial meningitis (van de Beek et al., 2006; Koedel et al., 2002, 2010). Leukocyte migration into the cerebrospinal fluid involves breaching the blood-brain barrier, and the resulting increase in permeability is the basis of brain oedema in meningitis (Gurney et al., 2006; Wispelway et al., 1988; Scheld et al., 2002). Vascular engorgement and hydrocephalus may also lead to intracranial hypertension, affecting global cerebral blood flow. Loss of autoregulation may further increase brain oedema through increased capillary pressure (Pedersen et al., 2008). In addition, vasculitis may induce focal reductions in blood flow. Septic chock and hypovolaemia from systemic inflammation is common (van de Beek et al., 2006; Maconochie et al., 2008). As in traumatic brain injury, intracranial hypertension and ischaemia are the major causes of death in meningitis, and the main therapeutic targets for supportive intensive care.

Microcirculation

Vessels with a diameter of less than 100 μm comprise the microcirculation, and the very smallest these, the capillaries with a diameter of 4–9 μm and the venules with a diameter of 7–30 μm , constitute the interface between blood and tissue. They are composed of a single layer endothelial cells and a surrounding basement membrane, and around the venules also a weak layer of smooth muscle cells.

As the arterial tree branches, the number of vessels and the cross-sectional area increase, while pressure and flow velocity decrease. At the capillary level, flow velocity is merely 0.3 mm/s – 1/1000 of the aortic systolic flow – and the flow is no longer pulsatile. The cross-sectional area has also increased by a factor 1000. The total surface area of the exchange vessels has been estimated to 500–1000 square metres. In most tissues, not all capillaries are open at each given moment. The smooth muscle layer in terminal arterioles contract spontaneously and divert flow to open capillaries. These precapillary sphincters relax in response to hypoxia, thereby acutely regulating capillary flow to meet tissue metabolic demands. Capillary recruitment also increases the total area for diffusional exchange, which is of importance for solute and solvent transfer. The number of open capillaries does not, however, influence total peripheral vascular resistance. The major pressure drop in the systemic circulation lies within the arterioles (Guyton and Hall, 2000; Levick, 2000). In the brain, all capillaries are considered to be constantly perfused (Kuchinsky and Paulson, 1992), due to the high metabolic demands of neurons and their inability store energy rich substrates as glycogen.

Regulation of pressure and flow

The hydrostatic pressure in the microcirculation is regulated through nervous, hormonal and local metabolic control to protect the tissue from high or low systemic pressures and to meet tissue demands. The capillary pressure, P_c , depends on the ratio of arterial and venous resistances (R_a/R_v), as described by Pappenheimer and Soto-Rivera (1948). Precapillary constriction, as for instance in haemorrhage, will decrease P_c and induce a transient absorption of interstitial fluid (see equation 2 below). In response to changes in transmural pressure, arterioles will contract or dilate to regulate the downstream pressure. This intrinsic myogenic activity is extremely important for the pressure autoregulation of the exchange vessels. The autoregulation of capillary pressure is present in most tissues, and it is especially efficient in the brain vasculature, where resistance is more segmental and up to 50% of the pressure drop takes place in larger arteries (Heistad et al., 1978; Guyton and Hall, 2000). During systemic inflammation, as in sepsis or after trauma, the myogenic control is probably lost, which may explain some of the plasma volume losses seen under such conditions, because of increased filtration pressure (Mellander et al., 1987; Dubniks et al., 2007;

Hollenberg and Cunnion, 1994). In brain trauma or meningitis, loss of vasoreactivity corresponds to the magnitude of the injury, and may have profound effects on capillary hydrostatic pressure and, leaving the microcirculation open to near systemic filtration pressures.

Microvascular flow is tightly regulated to meet the metabolic needs of the tissues – except for skin, where flow itself is part of temperature homeostasis, and kidneys, that are normally perfused at a constant level. Endothelial cells respond to frictional shear stress from the flowing blood by releasing vasoactive substances, causing relaxation of the vascular smooth muscle cells in a paracrine way. Circulating catecholamines and perivascular nerves affect resistance and flow in response to central stimuli. In the brain, there is a particular strong coupling of blood flow to metabolism. Hypoxia and hypercarbia are strong vasodilating stimuli, whereas hyperoxia and hypocarbia contracts resistance vessels (Levick, 2000, Guyton and Hall, 2000).

Regulation of permeability

The endothelial cells also regulate permeability by the contractile cytoskeleton. Through cell contraction or relaxation, the size of the interendothelial clefts can be modified. Pro-inflammatory substances induce contraction of the cytoskeleton through Ca^{2+} -dependent pathways. Factors raising levels of cyclic adenosine monophosphate (cAMP) relaxes the cells and counteracts the increase in permeability (Galley et al., 2004, Levick, 2000). There is extensive interaction between the endothelium and the immune and haemostatic systems. The normal endothelium acts anti-inflammatory and anti-coagulant, but is pro-inflammatory and pro-thrombotic in for example trauma and infection, making it a target for interventions in the critically ill (Galley et al., 2004).

Transvascular exchange

A semipermeable membrane, permeable only to solvent but not to solute, is the basis for osmotic flow between solutions of different concentrations. Fluid will cross the membrane from low to high concentration to even out the gradient. Fluid movement can be balanced by an opposing hydrostatic pressure. The pressure resulting in no flow will equal the osmotic pressure, π , of the solution. The osmotic pressure depends on the number of dissolved particles according to van't Hoff's equation:

$$\pi = CRT \quad (\text{Eq. 1})$$

where C is the millimolar concentration of the solute, R is the universal gas constant, and T is absolute temperature. At body temperature RT is 19.3 mmHg/mmol/L. With physiologic osmolality of about 300 mosm/L, the osmotic

pressure in plasma, in the interstitium, and in all cells will reach the tremendously high value of $19.3 \times 300 \approx 5800$ mmHg. As water and small hydrophilic solutes pass freely across the capillary membrane through clefts between endothelial cells, only plasma proteins (“colloids”), with a concentration of only 1 mmol/L, contribute to the effective osmotic pressure in plasma. Albumin, being the most abundant plasma protein, accounts for about 75% of the colloid osmotic pressure. The colloid osmotic pressure is about 30% higher than would be expected from protein concentration alone, because the negatively charged albumin attracts nearby cations – the Donnan effect – and is normally around 25 mmHg. (Levick, 2000; Holbeck, 2006). In the following π or COP will denote colloid osmotic pressure, and crystalloid osmotic pressure will be denoted P_{osm} .¹

There is a constant net filtration of plasma water over the capillary wall, cleared by the lymphatic drainage of the tissues. Plasma proteins are also lost at a rate of about 5–10%/hour (Marx, 2003): the transcapillary escape rate. Already in 1896, Starling postulated that the transcapillary colloid osmotic pressure of plasma proteins ($\Delta\pi$) counteracts the transcapillary hydrostatic pressure (ΔP) and that the balance determines fluid flow (J_v) over the exchange vessels (Starling, 1896). The equation bearing his name has later been derived from thermodynamics:

$$J_v = L_p S (\Delta P - \sigma \Delta \pi) \quad (\text{Eq. 2})$$

where $L_p S$ is the product of hydraulic conductivity (the pressure dependant water permeability) and total surface area available for exchange and σ is the reflection coefficient for proteins (Kedem and Katchalsky, 1958). σ is defined as the effective fraction of the calculated osmotic pressure in plasma. It has a value between 0 and 1 and describes the permeability of the osmotic solute (Staverman, 1951). For proteins, σ is typically > 0.9 in skeletal muscle and gut, and for electrolytes close to zero in most tissues. In the brain, however, σ for both proteins and electrolytes is 1 or close to 1, which is of profound importance for brain

¹In paper I and II, the terms COP and plasma oncotic pressure (POP) are used interchangeably, because of adherence to reviewers preferences.

volume regulation (Guyton and Hall, 2000; Levick, 2000; Levick and Michel, 2010; Rippe and Haraldsson, 1994; Fenstermacher, 1984).

Two-pore theory

Transport in major vessels is purely convective – this is indeed the evolutionary purpose of the circulatory system; life had outgrown diffusion distances – but at the capillary level, diffusion is by far the most important mechanism for exchange of nutrients and metabolic waste between tissue and blood. It has been estimated that diffusional exchange of water and nutrients is 80 times faster than plasma flow along the capillary (Guyton and Hall, 2000). 85–95% of water and small hydrophilic solutes leave the capillary through clefts between the endothelial cells, that effectively restrict permeation of macromolecules the size of albumin and larger. The existence of these small pores was suggested in 1951 by Pappenheimer, Renkin and Borrero, after experiments showing size selectivity of the endothelial barrier (Pappenheimer et al., 1951). Grotte subsequently introduced the concept of large pores, to account for macromolecular transport (Grotte, 1956).

Small pores are evenly distributed along the capillary and have a functional size of 4–6 nm (*i.e.* the size they would have, if they were cylindrical) and constitute only 0.1% of the total area of the exchange vessels. Macromolecules leave the microcirculation mainly by convection through 20–30 nm wide large pores, most of which are located at the venular end of the capillary. The ratio of large to small pores is estimated to 1/10 000–1/30 000, and at normal permeability, large pores account for 4–10% of the total fluid flow (Rippe and Haraldsen, 1994). Across the large pores, the colloid osmotic gradient, $\Delta\pi$, is practically zero, and only the hydrostatic pressure gradient drives the flow. Consequently, regardless of the net Starling forces across the capillary boundary, there is always a loss of protein and fluid to the interstitium. It follows that an increase in number of large pores, from inflammation, or an increase in hydrostatic pressure will increase the transcapillary filtration. (Levick and Michel, 2011). In states of systemic inflammation, as sepsis or trauma, this constant loss of plasma volume may compromise cardiovascular filling and performance.

In addition to pore size, the endothelial lining of negatively charged proteoglycans, the glycocalyx, contributes to the sieving properties of the barrier (Michel and Curry, 1999; Levick and Michel, 2010; Rippe and Haraldsson, 1994; Bansch et al., 2011). A third pore family, the aquaporins, with a diameter of less than 0.4 nm, account for only a few per cent of the total transvascular fluid exchange in most tissues.

An alternative pathway for macromolecules is transcytosis by vesicular uptake and transport. This theory has been questioned on theoretical and experimental grounds (Rippe et al., 2002), and probably does not explain bulk transfer of macromolecules in most tissues.

Nitric oxide

NO is mainly produced by NO-synthases (NOS) through oxygen dependent conversion of L-arginine to NO and L-citrulline. Three iso-enzymes are known: the inducible, the neuronal and the endothelial (iNOS, nNOS, and eNOS, respectively). Endothelial-produced NO is a central regulator of basal vascular tone in both brain and peripheral tissues. Factors raising intracellular Ca^{2+} -levels, like acetylcholine and shear stress, increase eNOS activity. NO diffuses quickly to vascular smooth muscle to cause an increase in cyclic guanosine monophosphate (cGMP), in turn lowering Ca^{2+} -levels and relaxing the cell (Faraci and Heistad, 1998). NO may also regulate basal microvascular permeability (Persson et al., 2003) and has antiaggregatory effects on thrombocytes (Moncada, 1997). In disease, NO is a dual sword. Excessive NO production in sepsis and trauma contributes to vasoplegic shock, and may increase vascular permeability in brain and body (Thiel and Audus, 2001, Moncada, 1997). NO produced by Ca^{2+} -independent iNOS in macrophages is a part of the non-specific immune response, and is cytotoxic to pathogens as well as host.

In brain trauma, reduced production of NO is implicated in microvascular dysfunction, microthrombosis, and blood flow reductions, and is a possible target for pharmacological intervention (Hlatky et al., 2003; Lundblad and Bentzer, 2007).

Prostacyclin

Prostacyclin (PGI_2) is the main arachidonic acid product in the endothelium, and has a short half-life of only 2–3 minutes. It is constitutively formed in endothelial cells, and its main physiologic action is considered to be platelet inhibition – it is the most powerful platelet inhibitor yet described – but it also regulates microvascular permeability and tone by increasing cAMP in endothelial and vascular smooth muscle cells after binding to its membrane bound receptor, the IP-receptor (Vane and Botting, 1995). Prostacyclin may also bind to nuclear peroxisome proliferator-activated receptors, PPARs, that are implicated in anti-inflammatory regulation and cytoprotection after ischaemia/reperfusion injuries (Chen et al., 2009). Clinically prostacyclin is mainly used as a vasodilator in pulmonary hypertension and peripheral artery disease and as an anticoagulant.

In sepsis and systemic inflammation, prostacyclin production may be reduced and the balance between prostacyclin and its physiologic counterpart, the vasoconstrictive and pro-coagulant thromboxane A_2 , disturbed (Zardi et al., 2007). In brain trauma, prostacyclin production may be insufficient for maintenance of microvascular function (Lundblad et al., 2007). Promising results of prostacyclin treatment have been presented in experimental brain trauma (Bentzer et al., 2001, 2003).

Statins

Statins are inhibitors of 3-hydroxymethylglutaryl-coenzyme-A reductase, the rate-limiting step in cholesterol synthesis. Their primary use as blood lipid lowering agents have made them international bestsellers after a number of studies showing secondary preventive effects on major cardiovascular events, such as myocardial infarction, angina pectoris, and ischaemic stroke (Pedersen et al., 1994; Sever et al, 2003). It is now well recognized that the effect of statins on vascular disease is beyond what can be explained by their lipid lowering effect alone (Adam and Laufs, 2008; Prinz and Endres, 2009).

Whereas other potential neuroprotectants have failed to progress beyond phase II clinical studies (Loane and Faden, 2010), statins have made it to adjuvant brain injury therapy through the backdoor. By widespread use in a stroke-prone population, the effect of statin pre-treatment could be elucidated. Two sides of the coin were discovered: statins up regulate endothelial NO production, but in statin withdrawal there is a strong rebound effect and suppression of eNOS (Prinz and Endres, 2009; Laufs et al., 2000).

In brain trauma, experimental evidence suggests improved microvascular function, reduced oedema, reduced microthrombosis, reduced oxidative stress and inflammatory cytokines, improved angiogenesis, increased neuronal survival and better neurological outcome after statin treatment (Lu et al., 2004, 2007; Wang et al., 2007; Mahmood et al., 2008; Chen et al., 2009; Beziaud et al., 2011).

In small, randomized clinical studies on statin treatment in subarachnoid haemorrhage and ischaemic stroke there are conflicting data regarding neurological outcome and incidence of vasospasm (Lynch et al., 2005; Vergouwen et al., 2009; Blanco et al., 2007; Montaner et al., 2008). In human brain trauma one small study suggests improved memory after statin treatment (Tapia-Perez et al., 2008).

By inhibiting the conversion of HMG-CoA to mevalonate, statins reduce the availability of isoprenoids. These short lipophilic molecules are necessary for the membrane-bound, active form of a number of small GTPases, including the Rho family, that control a vast number of cellular functions. This is held to be the principal mechanism by which statins exhibit anti-inflammatory, anti-oxidative, and positive endothelial effects separate from their cholesterol-lowering properties (Prinz and Endres, 2009; Rikitake and Liau, 2005). RhoA and the associated Rho kinase (Rho/ROCK pathway) negatively regulate eNOS activity, both by affecting eNOS mRNA stability and by inhibiting phosphorylation of eNOS to its active form. Rho kinase inhibition by statins therefore increases both the production and activity of eNOS. Furthermore, decreased production of reactive oxygen species (ROS) may affect the half-life of NO. Apart from the vascular effects, NO may also have direct effects on platelet aggregation (Laufs et al., 2000).

Fluid therapy

Intravenous fluids are used to maintain homeostasis of body fluid compartments, and as such they should be considered as drugs. As for all drugs, the benefits must outweigh the risks. Compared to many other drugs, fluids have a wide therapeutic range, as the healthy body possesses finely tuned mechanisms for keeping total body water within tight limits (Giebish and Windhager, 2003). In the critically ill, however, fluid therapy must be titrated. Tissue oedema from fluid overload is common and associated with increased morbidity (Marx, 2003; Arikan et al., 2011).

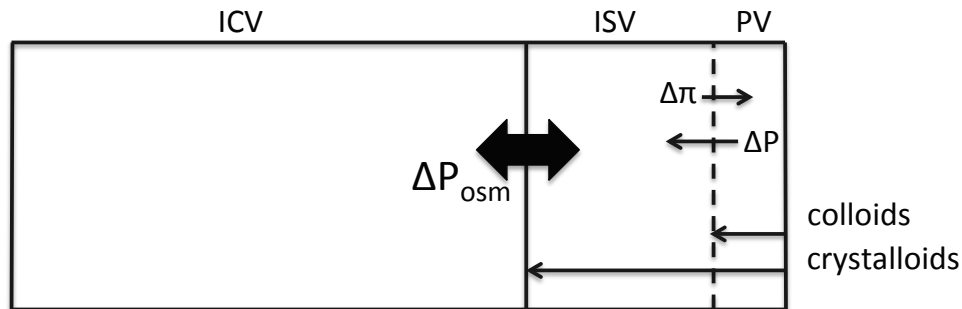


Figure 1. Schematic illustration of body fluid compartments. The intracellular volume (ICV) constitutes two thirds of the total body water. The extracellular volume is divided into interstitial volume (ISV) and plasma volume (PV). Only water passes freely between all compartments. A crystalloid solution introduced into the plasma volume will rapidly be distributed equally with the whole extracellular compartment (ICV + PV), whereas a colloid to a will remain in the PV to an extent depending on its permeation through the capillary barrier, the boundary between the PV and the ISV. The Starling equilibrium (equation 2) governs solvent flow over the capillaries, with hydrostatic and colloid osmotic pressure gradients of about 20–30 mmHg. The boundary between the ISV and ICV is the cell membrane, impermeable to even to small hydrophilic substances. Fluid flux over the cell membrane is governed the total osmolar gradient. The total osmotic pressure in each fluid compartment is 5800 mmHg – 200 times the Starling forces!

ΔP_{osm} = crystalloid osmotic gradient, ΔP = transcapillary hydrostatic pressure, $\Delta \pi$ = transcapillary colloid osmotic pressure.

Fluids used for plasma volume expansion can be divided according to the molecular weight (MW) of the solutes. Crystalloids, like saline and Ringer's solution, contain electrolytes (chiefly sodium and chloride) and sometimes buffer bases (acetate, lactate) with MWs spanning from 20–90 Da. They are small enough to pass freely through the small pores of the capillary barrier, and are therefore rapidly distributed in the whole extracellular fluid after intravenous administration. The plasma volume expansion is, at best, 20–25% of the infused volume. (Hillman et al., 1997, Persson and Grände, 2005). Colloids, on the other hand, are defined as solutions with MW > 30 kDa, and remain primarily within the vascular space. A portion of them, depending on the prevailing permeability and capillary hydrostatic pressure, will leak through the large pore system (Dubniks et

al., 2007, Rippe and Haraldsson, 1994). Synthetic colloids, made of starch, gelatin or dextran, are a mixture of molecular sizes. Part of the infused volume will then behave in a crystalloid way, with larger distribution to the interstitium and faster renal elimination. Synthetic colloids are also degraded relatively fast, which shortens their plasma half lives. In our studies we used albumin as colloid because of its longer lasting and predictable plasma expanding effect. Albumin is also considered the safest colloid in intensive care, because of minimal effect on coagulation, renal function, and with negligible risks of anaphylaxis (Hartog et al., 2011). The use of albumin in neurocritical care is, however, controversial, and this issue will be discussed in some depth below.

Volume regulation of the brain

The blood-brain barrier is the functional unit separating the brain interstitium from the rest of the circulation. It is composed of the capillary endothelial cells, the basal membrane, vascular pericytes, and the foot processes of astrocytes, and it is essential controlling the composition of the brain extracellular fluid. Paracellular passage of polar solutes is effectively hindered by tight junctions between the endothelial cells, and only water permeates the barrier passively. Specific transport systems regulate the entry of nutrients into the brain, and can exclude or actively efflux unwanted substances. Lipophilic substances, like oxygen, carbon dioxide and anaesthetics, diffuse freely, like in the rest of the body. When it comes to protect the brain from harmful substances, the blood-brain barrier thus performs poorly and without much discrimination. Alcohol, nicotine and other substances of abuse have free access to the mind, whereas therapeutic drugs, like penicillin, may be left outside (Abbott et al., 2006). Luckily, passage of the most dangerous substance of them all is effectively controlled: *water*.

The reflection coefficient, σ in equation 2, is practically 1 for both proteins and electrolytes at the blood-brain barrier. Consider the total effective osmotic pressure in all fluid compartments of about 5500 mmHg. Should there be even a minute transvascular filtration or absorption of water because of changes in hydrostatic or *colloid* osmotic pressure gradients, the *crystalloid* osmotic pressure in the brain interstitium would change immediately, by dilution or concentration, and terminate the fluid flux. Thus, the transcapillary hydrostatic and colloid osmotic pressures of about 25 mmHg have no influence on water movement into the brain. This osmotic buffering system is the basis for the strict volume control of the brain (Fenstermacher, 1984). It follows that isotonic crystalloids do not diffuse passively into the brain, which has been repeatedly shown experimentally (Zornow et al., 1987; Jungner et al., 2010). Likewise, a low colloid osmotic pressure in plasma, as in hypoalbuminemia or after large amounts of crystalloids, has no influence on brain water content because of the effective osmotic buffering

capacity of the blood-brain barrier. In other words, for the healthy brain, an isotonic crystalloid is the perfect plasma volume expander.

If the barrier is breached, however, as in trauma, infection, or ischaemia, (Hawkins, 2005; Krizbai et al., 2005; Abbott et al., 2006; Preston and Foster, 1997; Whispelway, 1988) the volume control fails. σ for electrolytes will be drastically reduced, and for proteins it may reach the value of peripheral vascular beds, if the holes in the barrier are not too large or too many. At such a state, the balance between transvascular hydrostatic and colloid osmotic gradients will govern fluid flow. Crystalloid solutions will diffuse passively over the barrier and increase brain water content. Colloid solutions, on the other hand, may still respect the barrier and thereby minimize brain oedema. Obviously, the hydrostatic capillary pressure will be an important determinant of transvascular fluid flow (Kongstad and Grände, 2001; Grände, 2006).

Brain oedema

Brain oedema, excess fluid in the brain, is classically defined as extracellular or intracellular depending on its main location (Klatzo, 1967, Unterberg, 2004; Kimelberg, 2004). Extracellular oedema is considered to be of vasogenic origin, where fluid is filtrated across an open blood-brain barrier. Note that vasogenic brain oedema is not necessarily rich in plasma proteins. Filtration can occur even at low permeability for macromolecules, as long as electrolytes pass unhindered and no crystalloid osmotic gradient can counteract the transcapillary hydrostatic pressure. The traditional animal model for studying vasogenic oedema has been the freeze lesion, which probably increases macromolecular permeability to a much larger degree than seen in trauma and infection (see further Models of disease in the Discussion). Therefore, the proper molecular size to probe the blood-brain barrier is not large proteins like albumin, horseradish peroxidase, or globulins, but smaller ones, like sucrose, EDTA or contrast agents like iohexol. Absence of extravasation of a large probe does not imply a functioning blood-brain barrier or a normal volume control of the brain!

Cellular oedema is the result of failing cell volume regulation. Stipulated mechanisms involve energy depletion and inability of the Na^+/K^+ -ATPase to maintain the transmembrane potential, increased permeability to Na^+ and K^+ , and cellular uptake of osmolytes, like the high levels of glutamate seen after brain trauma (Unterberg, 2004). Cellular oedema is most prominent in astrocytes, and may initially be manifest as a shift of water from the interstitium, without increased volume of the brain. (Kimelberg, 2004). The astrocytic perivascular endfeet express aquaporin-4, and these waterchannels have been implicated in the development of cellular oedema as well as the clearance of extracellular oedema. The mechanisms are still unclear, and the potential for treatment currently unknown (Bloch and Manley, 2007). It should be emphasized that cellular and

vasogenic oedema in no way are mutually exclusive, and that both types probably coexist in the traumatized brain (Marmarou, 2007).

Aims of the studies

- | | |
|------------------|---|
| Paper I | To compare the effect on crystalloid or colloid normovolaemic fluid resuscitation on brain oedema in a rat model of traumatic brain injury and haemorrhage, and to investigate if the observed difference could be explained by increased blood-brain barrier permeability. |
| Paper II | To compare intracranial pressure development after crystalloid or colloid normovolaemic fluid resuscitation in a cat model of bacterial meningitis. |
| Paper III | To investigate effects of prostacyclin on intracranial pressure and plasma volume in a cat model of bacterial meningitis. |
| Paper IV | To investigate potential effects of rosuvastatin treatment on brain oedema and microvascular function in a rat model of traumatic brain injury. |
| Paper V | To investigate if blood-brain barrier permeability is increased in pericontusional tissue early after traumatic brain injury in humans. |

Material and methods

Ethics

All animals used in study I–IV were purchased from professional breeders and treated in accordance with Swedish law. All experiments were approved by the Animal Ethics Committee at Lund University, Sweden.

The patients in study V were enrolled after informed consent from the next of kin, and, in the case of a favourable outcome, later from the patients themselves. The study was approved by the Human Research Ethics committee of Lund University, Sweden.

Anaesthesia and surgical preparation

Rats (I and IV)

In study I and IV, adult male Sprague-Dawley rats were used. Anaesthesia was induced by intraperitoneal injection of pentobarbital, and maintained with isoflurane in air on an open nose cone during vascular cannulation and preparation for trauma. The femoral artery was cannulated for monitoring of blood pressure and for blood sampling. The internal jugular vein was used for infusions and for measurement of central venous pressure (CVP) (Study I). Rectal temperature was maintained at a normal level through a feedback controlled heating pad. After trauma and interventions (see below), the animals were allowed to wake up in a temperature-controlled environment, and they were then transferred to their litter box with free access to food and water. At the time of the final measurements, the animals were re-anaesthetized with isoflurane in air in a closed chamber, vascular access was re-established and the trachea was intubated via a tracheotomy. The lungs were mechanically ventilated (Ugo Basile Animal Ventilators, Comerio, Italy) to an end-tidal carbon dioxide concentration of 4.5–5.5% (Capstar-1000; CWE, Ardmore, PA). After termination of the experimental procedures the animals were decapitated, and the brains were quickly harvested for the different analyses (see below).

Cats (II and III)

In study II and III, adult male cats were used. Anaesthesia was induced with an intramuscular injection ketamine, and maintained with α -chloralose after venous cannulation. The animals were ventilated with air via a tracheotomy using a volume-controlled ventilator with a positive end-expiratory pressure of 5 cm H₂O. End-tidal PCO₂ was continuously measured and kept at a normal level. Normal core temperature was ensured by a feedback controlled heating-pad. An arterial cannula was inserted for recording of mean arterial pressure and for blood sampling. The bladder was catheterized for urine volume collection. At the end of the experiments, the cats were killed by an infusion of potassium chloride.

Trauma model (I and IV)

Technical considerations

In the lateral fluid percussion technique (fluid percussion injury, FPI), focal brain trauma is induced by the propagation of a pressure pulse through a fluid filled cylinder and a distal tube directly onto the exposed dura. A pendulum is released from a calibrated height onto a freely moving piston at the proximal end of the cylinder. The transferred energy to the brain obviously depends on the area of the distal tube and the pulse pressure over time. The pressure is gauged in the distal tube. In the literature, by tradition, only the peak pressure is reported; therefore, the level of trauma in different studies cannot be directly compared. It is specific to each experimental setup and must be determined through other measures, such as histology or functional outcome. The recorded pressures vary little, however, once the FPI device is calibrated, and are useful as a marker of trauma intensity.

The fluid percussion setup is depicted in Figure 1. We used a trauma of approximately 2.4 bars, and the pressure pulse lasted about 21 ms. After some hesitation, it was experienced that this level of trauma numbs the tip of your finger for a short time without leaving marks or bruises.

Any factor affecting the pressure propagation will also affect the induced trauma. Especially, even minute bubbles of air entrapped in the distal tube, or larger amounts of air in the cylinder, will absorb some of the energy. It is therefore of utmost importance to ascertain an air-free system before each experiment. The pressure over time curve in our experiments was graphically displayed after digital conversion (AcqKnowledge 3.9.1; Biopac Systems, CA, USA), enabling a visual quality control of the trauma and an easy way of detecting air in the system.

During study I we experienced a series of animals with dural tears after trauma. The reason was found to be crystalline deposits of sodium chloride within the distal tube, probably affecting stroke length of the fluid column and/or peak pressure, thereby increasing the tension in the dura until it split. After this time

consuming and frustrating incidence, the system was thoroughly and regularly cleansed.

Protocol

Anaesthetized rats were positioned with the head in a stereotaxic frame, and the skull was exposed via a midline incision after infiltration of a local anaesthetic. A left-sided craniotomy was performed with a 5 mm trephine, midway between the coronal and lambdoid sutures. A plastic Luer cone, cut from a cannula to fit the distal metal tube of the FPI-machine (Amscien Instruments, Richmond, VA, USA), was fitted to the craniotomy with Histoacryl (Braun, Tuttingen, Germany). A metal screw was anchored in the frontal bone, and the Luer cone was secured to the screw with dental cement (Dentalon Plus; Heraeus Kulzer GmbH, Hanau, Germany). The plastic connector was filled with saline and connected to the distal tube of the FPI device, ensuring an air free system. After trauma, the connector was rapidly removed and the dura was carefully examined. Animals with dural tears were excluded from further study. A typical reaction to trauma included apnea and seizures lasting 10–30 seconds. In some cases, securing the airway by the “tongue thrust manoeuvre” or facilitating ventilation by manual compressions of the thorax was necessary.

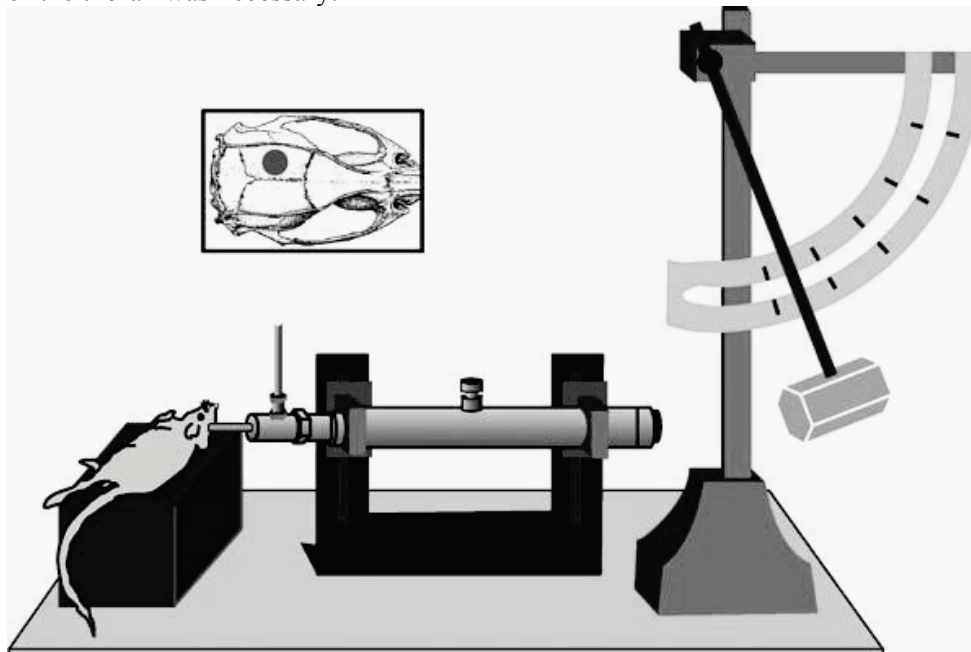


Figure 2. Fluid percussion injury device and the site of the trepanation. See text for details. Reprinted with kind permission from Thompson et al., 2005.

Meningitis model (II and III)

In the anaesthetized cats the dura was punctured at the level of the atlanto-occipital membrane, using a 22-gauge spinal cannula. 0.8×10^6 U/kg of *E. coli* derived lipopolysaccharide (LPS) in 0.5 mL saline was injected intrathecally. In this model, a clinical picture of severe meningitis is developed within a few hours, with marked plasma volume loss and increased ICP (Temesvári et al., 1993; Gärdenfors et al., 2004). At the time of injection, there is a transient peak in ICP – an effect of the injected volume – confirming correct placement of the needle.

Motor function (I)

In study I, the trauma intensity was evaluated with the composite neuroscore test (McIntosh et al., 1989, Bentzer et al., 2001). Before and 24 hours after trauma or sham injury the following parameters were evaluated, with scores from 0 (severely impaired) to 4 (normal): right and left forelimb and hindlimb provoked flexion; ability to resist right and left lateral pulsion; ability to stand on an inclined plane. Summation of the results generated the composite neuroscore (0–28).

Microdialysis and intracranial pressure registration (II and III)

In each parietal bone, a conical hole of 3 mm to 4 mm in diameter was bored about 12 mm lateral to the sagittal suture. When the skull was breached the dura was punctured with a cannula. A microdialysis catheter with an external diameter of 0.5 mm, a length of 10 mm, and a molecular cut-off of 20 kDa (CMA/20; CMA Microdialysis, Stockholm, Sweden) was placed in the cortex through the right bore hole. A tip transducer for intraparenchymal pressure registration (Codman and Shurtleff, Raynham, MA, USA) was placed in the cortex through the left bore hole. To prevent leakage of cerebrospinal fluid, the dura and the bore holes were sealed with Histoacryl. The microdialysis catheter was perfused with Ringer's acetate at 0.5 mL/min. The microvials collecting the dialysate were replaced every second hour, and stored at -30°C until analysis. Dialysate concentrations of glucose, lactate and pyruvate were performed with a CMA 600 (CMA, Microdialysis, Solna, Sweden).

Cerebral blood flow (IV and V)

Study IV

Cortical blood flow was measured with the autoradiographic technique described by Sakurada et al. (1978), using the following equation:

$$C_i(T) = \lambda K \int_0^T C_a e^{-K(T-t)} dt \quad (\text{Eq. 3})$$

where $C_i(T)$ is the tissue concentration of an inert, freely diffusible tracer; λ is the tissue:blood partition coefficient; C_a is the concentration of the tracer in arterial blood; t is time. K is defined as $K = (m/\lambda)(F/W)$, where F/W is blood flow per unit mass and m is a constant 0 – 1 describing the diffusive equilibration of the tracer between blood and tissue during the passage through the capillary. A value of m close to 1 implies that tissue uptake is not limited by diffusion, but by blood flow alone, and blood flow can be calculated as λK . For the tracer ^{14}C -iodoantipyrine $\lambda = 0.8$ and $m = 1$ (Sakurada et al., 1978).

In brief, anaesthetized rats, artificially ventilated to normocapnia, received an infusion of the tracer during 45 seconds, and 10 arterial blood samples were obtained at predefined intervals. At the time of the last blood sample, the animals were decapitated and the brains were rapidly frozen and later sectioned with a cryostat. The sections were exposed on an x-ray film together with calibrated standards. Tissue concentrations, $C_i(T)$, in the traumatized and contralateral cortex was determined by densitometry, after digital conversion of the developed films, using ImageJ, a public image analysis software from the National Institute of Health, US. Blood concentrations, $C_a(t)$, were measured in a liquid scintillation counter after quenching and bleaching. Blood flow was calculated as the mean of two levels, -4.5 and -6.8 mm relative to bregma (Bentzer et al., 2003).

Study V

Perfusion CT examinations were performed on a Brilliance 64 scanner (Philips Healthcare, Best, Netherlands) in patients and controls in study V. After initial non-contrast enhanced imaging, a bolus of iohexol 350 mg/mL was given intravenously, and 8 continuous axial slices with a thickness of 5 mm were obtained every 1.5 seconds during 50 seconds. Cerebral blood flow was automatically calculated with the commercial software package Brain Perfusion (version 1.2, Philips Medical Systems, Best, Netherlands) according to the central volume principle. In the algorithm, time-enhancement curves from tissue and the anterior cerebral artery are used to calculate cerebral blood volume (CBV) and

mean transit time (MTT) of the contrast agent. CBF can then be calculated for each voxel as CBV/MTT (Wintermark et al., 2001).

Number of perfused capillaries (IV)

Rats in cortical blood flow experiments were given a bolus of fluorescein isothiocyanate marked albumin (FITC-albumin) at the start of the tracer infusion. Sections adjacent to those chosen for blood-flow measurements were analyzed with a laser scanning confocal microscope (Zeiss LSM 510; Oberkochen, Germany) and digitized. Vessels with a diameter of 5–12 μm containing FITC-albumin were considered to be perfused capillaries. The image analysis was performed with dedicated software (Sigmascan; Systat Software, Erkrath, Germany).

Blood-brain barrier permeability (I, IV and V)

Permeability is often accused when plasma constituents are found on the wrong side of a barrier, but it is important to remember that other factors influence extravasation. For small solutes, transported across membranes mainly by diffusion, plasma flow and the surface area available for exchange are the major determinants of extravasation beside permeability. For larger molecules, convection is of increasing importance, and capillary pressure may have to be accounted for (Levick and Michel, 2010, Rippe and Haraldsson, 1994).

The transfer K_i of a solute from blood to brain is a function of plasma flow (FV), blood-brain barrier permeability (P) and the total area available for transvascular exchange (S), assuming there is no backflow from the tissue compartment (Fenstermacher et al., 1981), according to the following equation:

$$K_i = FV(1 - e^{-PS/FV}) \quad (\text{Eq. 4})$$

When permeability is low and plasma flow in the tissue is sufficiently high for a sustained concentration gradient from blood to tissue ($K_i/FV < 0.1$), K_i approximates PS , the permeability surface area product, with an error of $< 6\%$ (Blasberg et al., 1983).

Study I and IV

K_i for the small hydrophilic complex $^{51}\text{Cr-EDTA}$ (molecular weight 358 Da, study I and IV), and for the large molecule $^{125}\text{I-albumin}$ (molecular weight 69 kDa, study

I), was used to measure post-traumatic permeability changes of the blood-brain barrier, using the equation:

$$K_i = B / \int_0^T C_a(t) dt \quad (\text{Eq. 5})$$

where B is the amount of the tracer in the tissue, $C_a(t)$ arterial concentration and t is time (Fenstermacher et al., 1981). Anaesthetized and mechanically normoventilated rats received an intravenous bolus dose of ^{51}Cr -EDTA or ^{125}I -albumin, followed by a constant rate infusion of ^{51}Cr -EDTA or saline, respectively. Plasma concentration of the tracer was measured at regular intervals, and the animals were decapitated after 40 min (Cr-EDTA) or 60 min (albumin). Tissue plasma volume was determined with an intravenous bolus dose of either ^{125}I -albumin or ^{131}I -albumin, allowed to circulate for the last 4 min of the experiment, and tissue uptake of the tracer was calculated as tissue activity minus regional plasma activity.

Study V

K_i for the small hydrophilic contrast agent iohexol (molecular weight 821 Da) was determined by Patlak plot analysis, based on the same two-compartmental, unidirectional model of blood to brain transfer (Patlak et al., 1983). After perfusion CT examination, 8 additional scans were performed during 25 minutes. The images were exported to ImageJ and analysed with a dedicated macro. Tissue amount of the tracer, A_m , can be described according to the following equation:

$$A_m = V_p C_p(t) + K_i \int_0^T C_p(t) dt \quad (\text{Eq. 6})$$

where V_p is the initial volume of distribution of the tracer and C_p is plasma concentration. Plotting $A_m/C_p(t)$ versus $\int_0^T C_p(t) dt / C_p(t)$ at all time points yields a linear curve with the slope of K_i and an intercept representing V_p . Change in enhancement ($\Delta\text{Hounsfield units}$) is directly proportional to iohexol concentration, and was used to determine A_m and C_p . C_p was determined in large venous vessels to avoid partial volume effects associated with arterial vessels. By calculating K_i for each voxel, permeability maps of the whole slice were generated. K_i was also calculated in tissue regions of interest (ROI) surrounding

contusions. The ROIs were chosen in non-ischaemic regions, as guided by the perfusion images.

Blood chemistry (I–IV)

Analysis of blood gases, haematocrit and electrolytes in study I–IV was performed with i-STAT (Abbot Scandinavia AB, Stockholm, Sweden), a bedside point-of-care system with minimal blood requirements. Osmolality (study I) was measured with the freeze point method (Micro-Osmometer Model 210; Fiske Associates, Norwood, Massachusetts, USA). Oncotic pressure (study I) was measured with a 10 kDa cutoff membrane osmometer (Osmomat 050; Gonotec, Berlin, Germany).

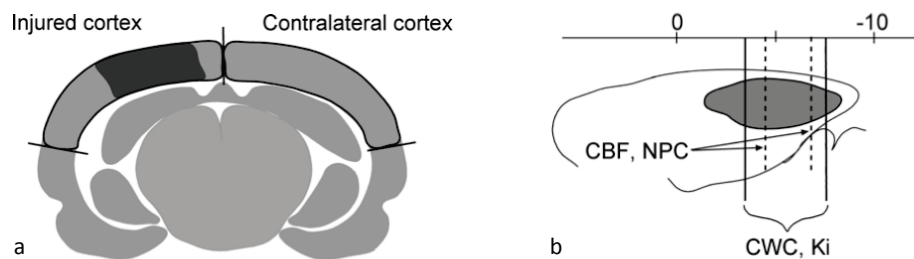


Figure 3. Study I and IV. Schematic illustration of the areas chosen for determination of cortical blood flow (CBF), number of perfused capillaries (NPC), cortical water content (CWC), and transfer constant for $^{51}\text{Cr-EDTA}$ (Ki) in coronal (a) and lateral (b) views. The shaded area represents the contusion.

Cortical water content (I and IV)

After decapitation, the brains were quickly removed and put on a chilled tray to minimize evaporation. A 4 mm slice was cut, centred over the contusion, and the cortex was dissected as shown in Fig X. Wet weights of the specimen were immediately determined, and after desiccation in 100 °C for 24 h, dry weight was obtained. Tissue water content (%) was calculated as $100 \times (\text{wet weight} - \text{dry weight}) / \text{wet weight}$. This is a simple procedure, widely used for determination of tissue water content, but rapid handling is of essence. The relative decay in water content is linear during at least 8 minutes, and was estimated to be 0.4–0.5%/minute (unpublished data, $n = 2$). Typical handling times from decapitation to completed weighing of all samples were 3–4 minutes. The evaporation during this time period must have increased the variation in water content data, but with equal magnitude in the different groups.

Plasma volume (I–III)

Plasma volume was estimated as the volume of distribution of ^{125}I -albumin measured five minutes after an intravenous injection. During this time, complete mixture within the plasma volume has been shown previously, and transcapillary escape is negligible, even in states of increased microvascular permeability (Persson and Grände, 2006). The technique is highly reproducible and is considered the gold standard for clinical measurements of plasma volume, provided that the amount of unbound iodine is low (Margaron and Soni, 2005, Valeri et al, 1973). The fraction of unbound iodine was $< 1\%$ in all experiments, measured as ^{125}I -activity in the supernatant after trichloric acid precipitation of the albumin and centrifugation. In study I, total blood volume was calculated as plasma volume/(1 – arterial haematocrit). Sample ^{125}I -activity was measured in a gamma counter (1480 Wizard; Wallace Sweden AB, Sollentuna, Sweden).

NO and prostacyclin (IV)

NO is rapidly oxidized to NO_2 (nitrite) and NO_3 (nitrate) in plasma, and the sum of these more stable molecules is commonly used as a measure of NO production (Tsikas, 2007). We used a commercially available kit (Cayman Chemical Co, Ann Arbor, Minnesota, USA), where plasma NO_3 is reduced to NO_2 and total NO_x is converted to an azo dye via the Griess reaction (Tsikas, 2007), which can be quantified photometrically with known standards. Prostacyclin also has a short biological half-life, but the concentration of the stable metabolite 6-keto-PGF $_{1-\alpha}$ is considered to reflect in vivo prostacyclin production (Fitzgerald et al., 1983). We employed a commercially available enzyme-linked immunosorbant assay (ELISA) kit for the detection of plasma 6-keto-PGF $_{1-\alpha}$ (Cayman Chemical Co., Ann Arbor, MI).

Fluids (I and II)

In study I, the osmolality of Ringer's acetate was increased to 296–300 mmol/L by adding concentrated NaCl. The 5% albumin was prepared from 20% solution diluted with Ringer's acetate and then adjusted to the same osmolality by adding concentrated NaCl. The rationale behind this modification was the need of iso-osmolar solutions, compared to rat plasma, without the large chloride load of normal saline.

In study II, normal saline and 20% albumin were used. There was no need to modify osmolality or buffer capacity of the fluids, as normal saline is indeed almost physiologic in the cat.

Experimental protocols

Study I – Fluid resuscitation in rat brain trauma and haemorrhage

Brain oedema was measured after 3 and 24 hours in rats subjected to fluid percussion injury and severe haemorrhage in groups randomized to resuscitation with either crystalloid or albumin. The lost blood volume of 20 mL/kg was replaced with 20 mL/kg 5% albumin (group A20), 50 mL/kg crystalloid (group C50), or 90 mL/kg crystalloid (group C90). Large and small molecular blood-brain barrier permeability was measured after trauma, to investigate the pathophysiologic basis of oedema development. To determine injury severity, motor function was evaluated blindly in separate animals subjected to trauma or sham trauma, but no haemorrhage or fluids.

Study II – Fluid resuscitation in cat meningitis

Intracranial pressure development was examined in the cat meningitis model. Four hours after intrathecal LPS-injection, the cats were randomized to a 6-hour infusion of normal saline (7.5 mL/kg/h) or 20% albumin (0.4 mL/kg/h) to reach normovolaemia (n = 7 in each group), or no fluids (n = 6). Intracerebral microdialysis was used to investigate signs of anaerobic metabolism and ischaemia. Plasma volume was measured at baseline, after 4 hours and at the end of the experiment.

Study III – Prostacyclin in cat meningitis

Intracranial pressure, plasma volume loss, and cerebral metabolic parameters were investigated in the cat meningitis model. Two groups (n = 7 in each group) were randomized to post-treatment with prostacyclin 1 ng/kg/min or vehicle. Cerebral metabolism was evaluated with the microdialysis technique. A sham group received intrathecal saline and vehicle (n = 3). All animals received an intravenous infusion of saline at 2.5 mL/kg/h.

Study IV – Statin treatment in rat brain trauma

Rats subjected to fluid percussion injury were randomized to 10 mg/kg rosuvastatin or vehicle given intravenously. 4 or 24 hours after trauma, the effects of treatment on brain oedema, cortical blood flow, blood-brain barrier permeability and capillary patency were investigated.

Study V – Blood-brain barrier permeability in human brain trauma

Between December 2006 and May 2010 patients presenting with evidence of severe traumatic brain injury were screened for eligibility, if a member of the research team was available. Inclusion criteria were suspicion of severe brain injury (GCS < 8) or contusions on initial CT, age 16–75 years, informed consent and a clinical indication for a perfusion CT. Exclusion criteria were need of immediate intervention, pregnancy, and lack of informed consent. Three control patients with lateralized brain tumour were included; one was excluded due to motion artefacts. Relevant clinical data was recorded. Blood-brain barrier permeability was calculated offline, without any impact on care.

Main results

Study I – Fluid resuscitation in rat brain trauma and haemorrhage

Trauma resulted in brain oedema and loss of motor function. Haemorrhage and resuscitation alone did not result in brain oedema (sham C90 group). Blood-brain barrier permeability was increased following trauma with a factor 50 for Cr-EDTA, and for albumin with a factor 10, with a decline from 3 to 24 hours. The permeability to albumin in non-traumatized muscle was of the same magnitude as in traumatized brain cortex. Fluid resuscitation in group A20 and C90 resulted in near normovolaemia at 3 hours, whereas group C50 was still hypovolaemic. Colloid osmotic pressure was maintained in the albumin group, and reduced by 35–40% in the crystalloid groups at 3 hours. At 24 hours, all groups had compensated plasma volume and colloid osmotic pressures, but haematocrit was still at post-resuscitation levels. Brain oedema was significantly lower in the A20 group compared to the C90 group at 3 and to both the C50 and C90 group at 24 hours.

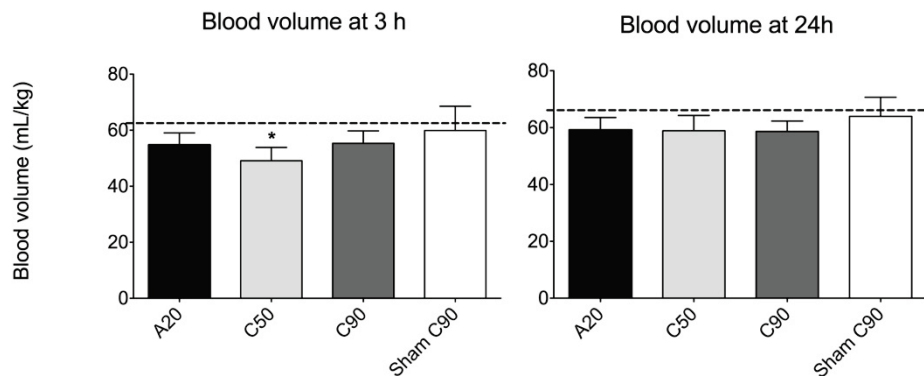


Figure 4. Blood volume at 3 and 24 hours after trauma, haemorrhage and resuscitation. A ratio of crystalloid:albumin of 4.5:1 resulted in equal volume expansion, approaching euvolaemia. The dashed lines represent mean baseline values for all groups. * $p < 0.05$ compared to A20 and C90 (ANOVA).

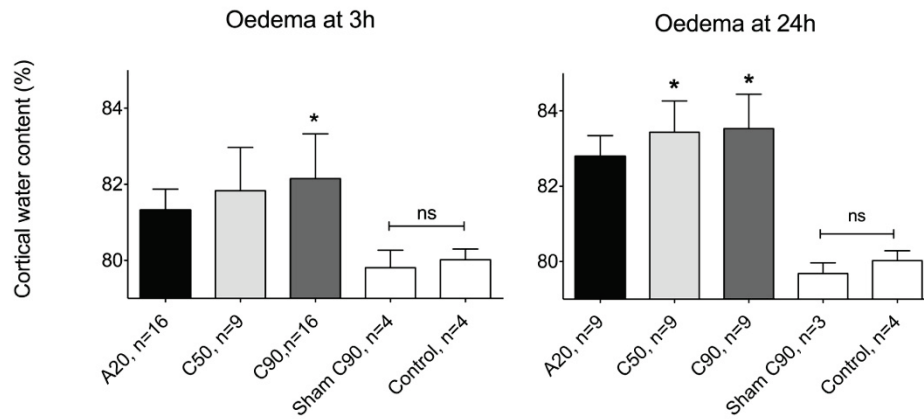


Figure 5. Brain oedema in trauma, sham-trauma, and control groups at 3 and 24 hours. Note the absence of oedema formation in the sham C90 group. Even a large volume load of isotonic crystalloid will not increase cortical water content because of the intact osmotic buffering capacity of the normal blood-brain barrier. * $p < 0.05$ compared to A20 (ANOVA), ns = non significant

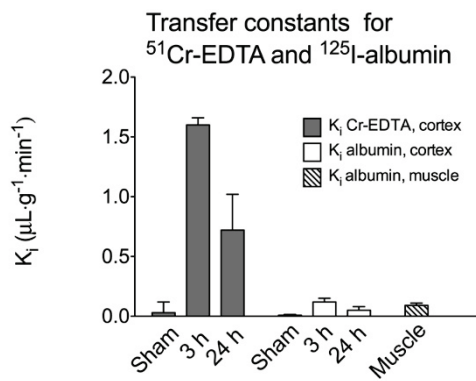


Figure 6. Blood to brain transfer constants, reflecting permeability, after trauma. Small molecular permeability (Cr-EDTA, molecular weight 358 Da) was increased up to 50-fold. Permeability to albumin (molecular weight 69 kDa) was in the range of values measured in normal muscle. These results indicate that the colloid osmotic gradient over the traumatized blood-brain barrier can be maintained, whereas the important crystalloid osmotic buffering capacity is lost. Note also the size selectivity of the increase in permeability, implying pore like opening of the BBB rather than increased vesicular transport. All changes significant compared to sham (ANOVA).

Study II – Fluid resuscitation in cat meningitis

Intrathecal injection of LPS induced an increase in ICP from baseline values of about 10 mmHg to about 20 mmHg after 4 hours. At this time point, the cats had also developed a sepsis-like condition with plasma volume depletion, tachycardia and hypertension. Between 4 and 10 hours, when fluids were given to restore plasma volume, ICP continued to increase in the saline group, but in the albumin group it stabilized or even tended to decrease. In the no fluid group, ICP reached a plateau. In both the saline and the albumin group, plasma volume was restored at

the end of the experiment. The cats in the no fluid group showed progressive haemodynamic deterioration, with declining mean arterial pressure and cerebral perfusion pressure, and a continuous loss of plasma volume. The difference in ICP between the albumin and the saline groups was highly significant during the last four hours of the experiment. In all groups, microdialysis data (lactate/pyruvate ratio and glucose) were within normal limits, indicating adequate cerebral blood flow and oxygenation.

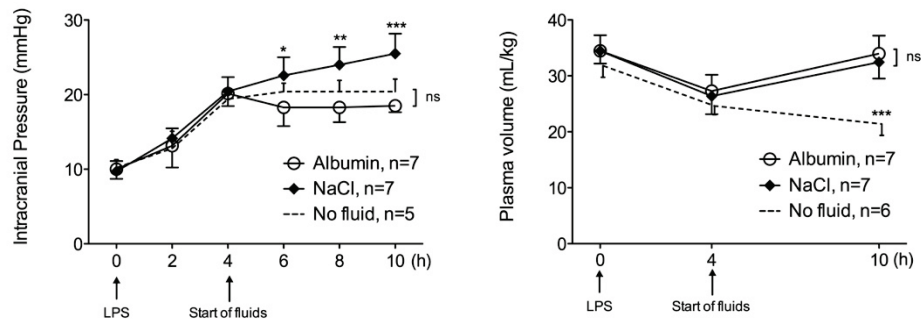


Figure 7. Changes in ICP and plasma volume. In both the saline and the albumin groups, euvoemia was reached at the end of the experiment, but at the cost of a steadily rising ICP in the saline group. Data are given as means and SD, or means only (sham group). * $p < 0.05$, ** $P < 0.01$, *** $p < 0.001$, ns = non significant (2-way ANOVA for repeated measurements)

Study III – Prostacyclin in cat meningitis

After LPS injection, ICP increased and plasma volume decreased to the same degree as in study II. Infusion of low dose prostacyclin induced a plasma volume recovery and an ICP plateau, whereas in the vehicle group ICP continued to increase and there was a further loss of plasma volume. The differences in plasma volume and ICP were significant at the end of the experiment. Diuresis was significantly higher in the prostacyclin group. In the sham group, no signs of meningitis or systemic inflammatory reaction were observed. As in study II, no signs of metabolic perturbation were observed in microdialysis data, indicating adequate supply of blood and oxygen.

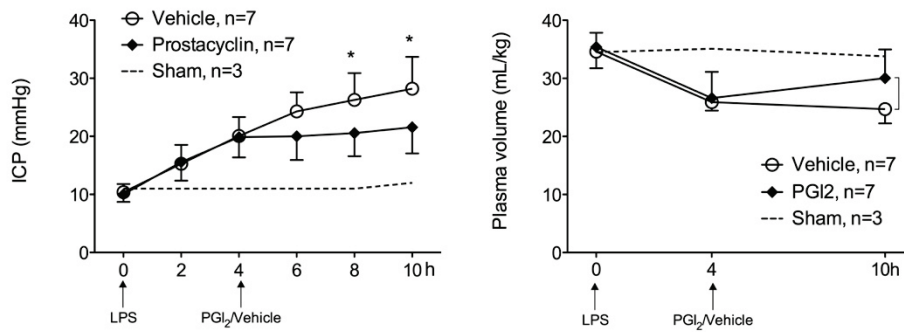


Figure 8. Treatment with prostacyclin resulted in a lower ICP and in plasma volume recovery, as compared to the vehicle group. Data are given as means and SD, or means only (sham group). * $p < 0.05$ (2-way ANOVA for repeated measurements (ICP); t-test (PV)).

Study IV – Statin treatment in rat brain trauma

Fluid percussion brain injury induced brain oedema and increased K_i for Cr-EDTA to the same degree as in study I. Trauma caused a decrease in cortical blood flow and number of perfused cortical capillaries. Treatment with rosuvastatin increased systemic bioavailability of nitric oxide, but decreased levels of prostacyclin. Mean arterial pressure was lower in the rosuvastatin group. There were no treatment effects on brain oedema, cortical blood flow, or K_i for Cr-EDTA, but there was a recruitment of cortical capillaries in the statin group, reaching significance at 24 hours post trauma.

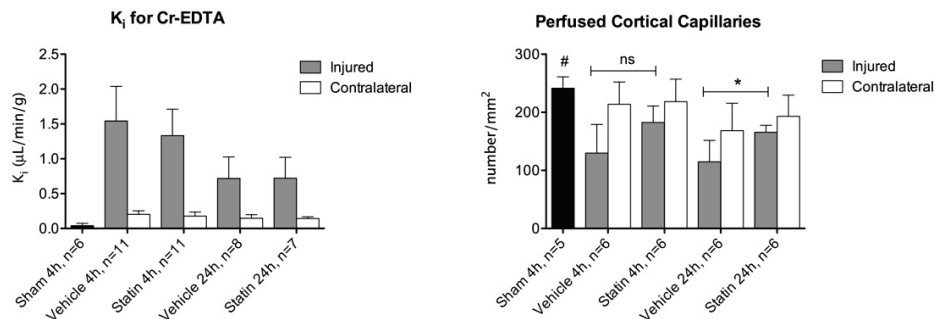


Figure 9. K_i for Cr-EDTA and the number of perfused cortical capillaries. K_i was not affected by statin treatment, but a small effect on permeability is still possible, considering that capillary

recruitment should increase the total area for diffusional exchange. # $p < 0.05$ compared to all contusion areas (ANOVA) * $p < 0.05$ (ANOVA)

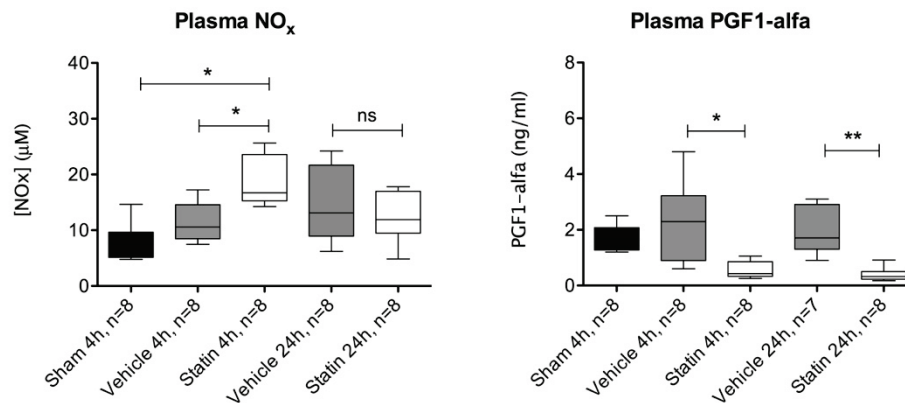


Figure 10. Metabolites of NO and PGI₂ in plasma after trauma and pharmacological interventions. Our results indicated increased overall production of NO and PGI₂ after trauma. Rosuvastatin treatment augmented NO production, probably through increased eNOS activity (see text for details). Contrary to our expectations, PGI₂ production was downregulated by rosuvastatin. Box and whiskers depict medians, interquartile range and range.

Study V – Blood-brain barrier permeability in human brain trauma

Seventeen patients were included (6 female), with a median age of 47 years (range 23 –70). Nine were involved in motor vehicle accidents. One patient suffered from multiple injuries, necessitating urgent, life saving abdominal and vascular surgery. The remaining patients suffered isolated head injuries. Four patients died because of intracranial complications. One patient remained in a vegetative state. One patient recovered to a pre-injury dependant state. Eleven patients survived with good recovery.

In control patients, K_i for iohexol was found to be about 0.06 ± 0.03 mL/min/100g, and in normal appearing tissue in trauma patients K_i was 0.08 ± 0.03 mL/min/100g. In areas surrounding contusions, K_i was $0.15 - 1.0$ mL/min/100g. In eleven of the seventeen patients, K_i was significantly higher in pericontusional tissue than in normal areas or controls. Six of the seven patients with an intracranial pressure of 20 mmHg or higher had increased K_i in pericontusional areas.

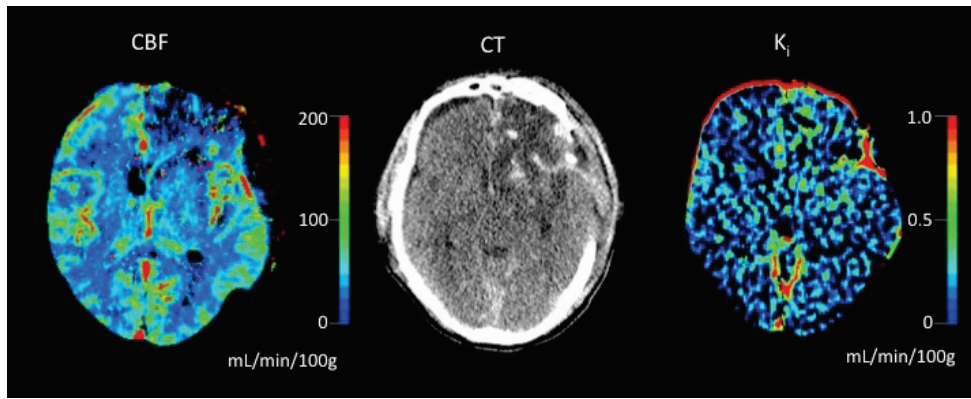


Figure 11. Perfusion- and K_i -maps (left and right panels) at day 5 after trauma in a patient with frontal contusions and craniectomy after surgical evacuation of haemorrhagic contusions and subdural haematoma. The patient suffered sustained intracranial hypertension, and later died because of massive infarction. Middle panel shows the corresponding contrast enhanced CT examination. Increased K_i can be seen around the left frontal area of contusions and ischaemia.

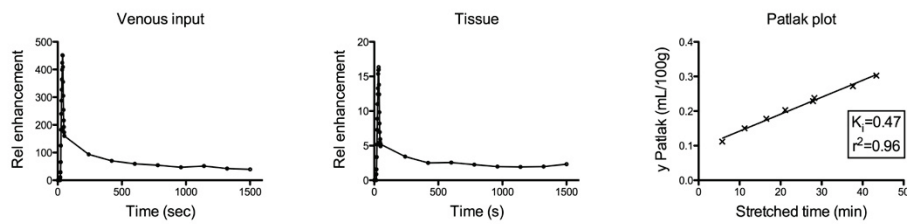


Figure 12. Representative measurements from patient 5 (see paper V in appendix), with relative contrast enhancement in tissue and vessels. First pass data are used for CBF calculation. In the Patlak plot the eight last data points are used. Note the constant and high gradient from blood to tissue. Because of the low uptake in cerebral tissue, arterial and venous concentrations of the tracer can be considered equal, but as veins are larger, they provide a more reliable source of measurement. The graphical display of the regression line enables an easy quality control of input data. Linearity is necessary in the Patlak model.

Discussion

The main results from the studies presented in this thesis is that the protective nature of the blood-brain barrier in keeping brain volume within rigid limits is lost following trauma or infection, and that this altered physiology may have wide implications for the care of patients with brain oedema and raised intracranial pressure.

Models of disease

To study disease in a laboratory setting, the models used – be it cell cultures, organs or whole animals – must bear some resemblance to the pathophysiological conditions at focus. The LPS induced meningitis mimics important aspects of bacterial meningitis in humans: it triggers the innate immune system through activation of Toll-like receptors (TLR-4), like in gram-negative sepsis (for a recent review, see Parker et al., 2007), resulting in opening of the blood-brain barrier and a systemic inflammatory response with general loss of plasma volume in a predictable and relatively fast way (Temesvári et al., 1993; Wispelway et al., 1988; Jungner et al., 2009). Therefore, it is a useful tool to explore the dynamics of intracranial hypertension and the sepsis features of meningitis. The cat is also large enough for reproducible measurements of ICP and for intracerebral microdialysis. Obviously, nothing can be learned from LPS induced meningitis concerning causal therapy or human outcome.

The fluid percussion injury has been extensively used for more than 20 years, and defining features of human brain trauma are reproduced (Thompson et al., 2005). Importantly, regarding aspects on fluid therapy, the permeability changes of the blood-brain barrier after fluid percussion injury are probably of the same magnitude as in human brain trauma. Thus, permeability for small polar substances is greatly enhanced, whereas the semipermeable properties of the blood-brain barrier to proteins are more or less sustained (Jungner et al., 2010; Lescot et al., 2006; Hillman et al., 2005; Kirchoff et al., 2008). This is in contrast to the freeze lesion model of brain oedema, where all attempts to maintain a colloid osmotic gradient from blood to tissue have failed, probably because of a large decrease in the reflection factor, σ , of macromolecules (Zornow et al., 1988; Kaieda et al., 1989 a,b; Zhuang et al., 1995).

Fluid management (paper I and II)

What is normovolaemia?

In our animal models of traumatic brain injury and bacterial meningitis, we wanted to mimic clinical aspects of hypovolaemic shock to necessitate fluid replacement. Our endpoints were plasma volume expansion to baseline intravascular volume, and in some sense this must represent a normovolaemic state. This is not to be confused with optimal preload conditions for the heart. Vasodilation, from sepsis or influence of anaesthetics, decreases the central blood volume even in the absence of plasma volume losses (Imm and Carlsson, 1993). Blood is pooled in the periphery, and compensatory mechanisms maintain perfusion by increasing heart rate and contractility. If true hypovolaemia occurs, from haemorrhage or internal fluid shifts, shock progresses, and perfusion of vital organs as brain and heart is preserved at the cost of flow to splanchnic organs, skin and muscles. Modern goal directed fluid therapy aims at optimizing cardiac preload, while keeping potential negative effects of the infusions at a minimum (Bundgaard-Nielsen et al., 2007; Reuter et al., 2002). In titrating fluid replacement, the bedside clinician is often left to indirect signs of cardiovascular performance, such as pulse, arterial and central venous blood pressures, capillary refill, or organ function. At best, more or less invasive monitoring may provide indices of the major determinants of stroke volume: preload, afterload and contractility. Direct measurement of plasma volume is rarely done, and baseline values are never known.

Maybe this explains why most clinical as well as experimental studies comparing different fluid therapies with haemodynamic endpoints end up with a ratio of crystalloid to colloid of 1–2.5:1, which is significantly less than the theoretical distribution of the different fluids (Drummond et al., 1998; Finfer et al., 2004; Myburgh et al., 2007; Brunkhorst et al., 2008; Hartog et al., 2011). When plasma volume expansion has been measured with a reference method in animal models (Persson and Grände, 2005; Jungner et al., 2010), or in patients (Lamke and Liljedahl, 1976), the volume expansion of different colloids has been 3–5 times that of crystalloids.

Pressures are easily gauged, but they are poor measures of volume and flow. Any outcome measure of drugs not given in “equipotent” doses regarding their primary effect must be skewed. As a parallel, morbidity and mortality effects of hypertensive drugs are measured at comparable blood pressures. Plasma volume expansion inferred from haemodynamic endpoints can never be accurate.

Results of study I and II

In study I and II, we were not primarily seeking to optimize circulation with fluid therapy; we wanted to investigate the major negative effect of fluid therapy – tissue oedema – at comparable doses of crystalloid or colloid. Reaching baseline

intravascular filling after induced hypovolaemia then would represent “equipotent” volumes. We believe that the cerebral oedema formation by crystalloid fluid replacement, manifested as increased cortical water content in study I and increased ICP in study II, can largely be explained by two mechanisms. Firstly, the breached blood-brain barrier after trauma or infection offers little restriction to small solutes, and crystalloid fluid volume is therefore spread by diffusion in the whole brain extracellular space (see Figure XX). Secondly, once the initial redistribution has occurred, plasma osmotic pressure will be lowered by dilution, and an imbalance of the Starling equilibrium (equation 2) may induce further filtration. Albumin infusions, on the other hand, remain to a large degree in the plasma volume, maintaining colloid osmotic pressure while minimizing redistribution to the interstitium. The hyperoncotic albumin solution used in study II was shown to expand plasma volume to the same degree as the 18 times larger volume of saline. Clearly, lost plasma volume was reabsorbed from the interstitium. Probably the same mechanism tended to lower ICP in the albumin group, although this was not significant in this small study.

The K_i for albumin in cortical tissue after trauma (study I, Figure XX), was of the same magnitude as in normal skeletal muscle, implying that the colloid osmotic gradient can be preserved in the injured brain..

Our results on ICP and plasma volume expansion in study II suggest that large volumes of crystalloids may have detrimental effects on ICP in severe meningitis, and that hyperoncotic albumin can be used for volume resuscitation without adverse effects on ICP. To increase plasma volume to the same degree with 5% instead of 20% albumin, a four times larger volume would have been needed. In essence, this would be equivalent to parallel infusions of normal saline and 20% albumin. Would this have increased ICP compared to 20% albumin alone? Probably not by much. One could speculate that the extra saline would have remained in the plasma volume in replacement of the otherwise absorbed interstitial fluid. The trend towards a decrease in ICP would probably not be seen though, for the same reason. In a clinical setting, with continuous plasma volume losses for longer periods, the effect on brain oedema might be more pronounced.

The steadily increasing ICP in the saline group is compatible with an open blood-brain barrier, highly permeable to crystalloids but not to macromolecules. The balance between the transcapillary hydrostatic pressure and the colloid osmotic pressure will then govern fluid flow across the barrier. Our results are compatible with the effects of induced hypertension earlier reported on the same meningitis model (Kongstad and Grände, 2001).

The no fluid group deserves some extra comments. The plateau in ICP development should not be interpreted as a protective effect of fluid restriction, even though microdialysis data did not show any signs of ischaemia. The cats in the no fluid group were deteriorating the last hours of the experiment, with oliguria, tachycardia and progressive hypotension. It is reasonable to assume

significant hypoperfusion of splanchnic organs, and that cerebral ischaemia would eventually occur.

Albumin in neurocritical care

For decades the debate on colloid or crystalloid fluids for plasma volume expansion has roared. In 1998, a metaanalysis reported a 6% increase in absolute risk of death with the use of albumin (Cochrane, 1998), and practically overnight, the use of albumin in adult intensive care was banned. The results were refuted by a subsequent larger metaanalysis (Wilkes and Navickis, 2001). In 2004, the Saline versus Albumin Fluid Evaluation Study (SAFE) was presented, a randomized, multicentre study enrolling almost 7000 patients, where the investigators were able to blind the clinicians to the study fluid. Primary outcome was death within 28 days, and no difference was reported between the study groups for the whole cohort. (Finfer et al., 2004). In trauma patients, however, there was a trend towards higher mortality in the albumin group, and a post-hoc follow up study (SAFE-TBI) at 24 months of 460 brain trauma patients revealed an almost doubled relative risk of death in the albumin group (Myburgh et al., 2007).

The SAFE-TBI results have gained widespread acceptance, and albumin therapy in traumatic brain injury has been questioned by many authorities (Caironi and Gattinoni, 2009; Alderson et al., 2011; Hartog et al., 2011). But the SAFE-TBI has also been criticized on many grounds (Powner, 2011; Grände, 2008). It has been argued that the cohorts, although equal enough to pass statistical scrutiny, were not perfectly matched; the albumin group being slightly older, with slightly higher ICP on admission, and with a tendency of more severe head injury scores (Drummond et al., 2011; Grände, 2008). It is also evident from the data that the excess mortality in the albumin group was *not* caused by intracranial complications, and it has been suggested that not albumin itself, but in combination with pharmacologically induced supra-physiologic blood pressures may explain the outcome (Grände, 2008). Furthermore, several single centre outcome studies on the Lund concept, with albumin as an integer component of oedema therapy, have reported equal or better outcome than comparable centres (Eker et al., 1998; Naredi et al., 1998; Naredi et al., 2001; Rodling et al., 2005; Rodling et al., 2009). A randomized study from Japan on brain trauma patients showed improved outcome and decreased brain oedema with albumin therapy compared to standard care (Tomita et al., 1994). Albumin use is also supported by studies on stroke (Tone et al., 1994; Palesch et al., 2006) and subarachnoid haemorrhage (Suarez et al., 2004), as well as numerous animal studies on different brain insults (Belayev et al., 2001; Belyayev et al., 1999; Ginsberg et al., 1999). Hypoalbuminemia in brain trauma patients has also been associated with worse outcome (Bernard et al, 2008).

In summary, there are strong physiological arguments in favour of albumin use in brain trauma patients. The value of preclinical physiologically controlled

studies and of single centre cohort studies is sadly overlooked in larger meta-analyses.

Cerebrovascular protection (paper III and IV)

Prostacyclin

In study III we showed positive effects of low dose prostacyclin on ICP, plasma volume, and diuresis in cats with LPS induced meningitis. As in study II, no effects on brain metabolism were seen. Probably, the 10-hour observation period was too short for ischaemia to occur. The systemic recovery of plasma volume must be explained by a shift in the Starling equilibrium (equation 2). Two possible mechanisms may contribute to such an effect: a true reduction of permeability, as has been shown in cat skeletal muscle after trauma (Persson et al., 2003; Möller and Grände, 1999), or an effect on capillary hydrostatic pressure (Dubniks et al., 2007). Systemic blood pressure did not differ between the groups, but it could be speculated that the anti-inflammatory properties of prostacyclin may have restored some of the lost autoregulatory capacity. The positive effect on diuresis confirms clinical reports of improved renal function after prostacyclin treatment in cardiac surgery (Morgera et al., 2002). The improved diuresis could be explained as merely an effect of the increased plasma volume, but it is tempting to suggest improved microvascular function also in the kidneys (Nielsen et al., 1997; Finn et al., 1987).

The ICP data could also be explained by a reduced permeability, but a permeability effect of prostacyclin has never been shown in brain circulation. On the contrary, prostacyclin, being a vasodilator and inhibitor of platelets, may increase the capillary surface area and thereby increase oedema and transfer of solutes (Bentzer et al., 2003). The lower ICP in the prostacyclin group may instead be explained by a lower hydrostatic capillary pressure. Again, it could be speculated that the anti-inflammatory effects of prostacyclin may have improved the myogenic autoregulatory response in precapillary sphincters. Further, vasoconstriction in response to prostacyclin has been shown *in vitro* in large cerebral arteries in the cat (Uski et al., 1983). The lower ICP may then be explained partly by an effect on blood volume and not on brain oedema alone. In piglets, on the other hand, prostacyclin appears to induce a marked vasodilation in LPS induced meningitis, with deleterious effects on cerebral haemodynamics and metabolism (Gärdenfors et al., 2004).

In summary, there seem to be important species differences in the effect of prostacyclin on cerebral vessels, and the results of our study should be interpreted cautiously. Low dose prostacyclin is currently sometimes used as an adjuvant to improve microcirculation in meningitis and traumatic brain injury (Grände, 2006), but always with continuous measurement of ICP. In trauma patients, there are case

reports supporting its use (Grände et al., 2000; Reinstrup and Nordström, 2011), and in a series of patients suffering from severe meningitis no adverse effects were found (Lindvall et al., 2004).

Rosuvastatin

As mentioned above, the drawback of capillary recruitment after brain trauma is the risk of increased transport of water and solutes over the increased capillary surface area, resulting in increased oedema and ICP. In that light, our result in study IV of increased capillary patency without adverse effect on K_i or oedema seems promising, and early effects on microvascular function by statin treatment may in part explain outcome effects reported by others (Chen et al., 2007; Lu et al., 2004; Lu et al., 2007). As hypothesized, we found evidence of increased NO availability after rosuvastatin, probably an effect of Rho kinase inhibition (Prinz and Endres, 2009). Theoretically, capillary recruitment in brain trauma can be referred to two mechanisms: decreased microvascular thrombosis and dilation of precapillary sphincters. Both mechanisms could involve NO stimulation.

The main source of substrate for endothelial prostacyclin synthase is considered to be COX-2 (McAdam et al., 1999). Given the promoting effects of statin treatment on COX-2 and PGI₂-synthase in models ischaemia/reperfusion injury (Birnbaum et al., 2007), we were expecting an increase also in prostacyclin. To our surprise, we found the opposite – a marked suppression of the metabolite PGF_{1- α} . Similar results have been reported in cell cultures and a model of atherosclerosis (Hernández-Prosa et al., 2002). Perhaps the diverging results depend on pathophysiological aspects of the models, or perhaps dose dependent statin effects on prostacyclin synthesis is not linear. Our dose was chosen to block HMG-CoA-reductase completely, and compared to other regimens reported in the literature it was in the high range ()

In the near future we will probably see clinical studies on statins as neuroprotectants, and perhaps also in other critical illnesses. A word of caution is warranted though: it is inconceivable to imagine solely salutary effects of blocking a rate-limiting synthetic step affecting such a multitude of cellular functions. The safety profile of statins in the critically ill may very well differ from the general public. For instance, co-enzyme Q10, an essential part of complex 1 in the mitochondrial electron transport chain, is also a mevalonate pathway intermediate (Hargreaves et al., 2005), and it is reduced by statin treatment. This could theoretically be harmful in an intensive care setting (Donnini et al., 2011).

Blood-brain barrier in human brain trauma (V)

There is ample evidence from animal models of traumatic brain injury that increased permeability of the blood-brain barrier coincides with the development of brain oedema (Jungner et al., 2010; Bentzer et al., 2003; Lundblad et al., 2004; Tanno et al., 1992). There are also strong proponents for the view that post-

traumatic brain oedema is mainly cellular and that the opening of the blood-brain barrier is transient and of little importance (Barzó et al., 1997; Stroop et al., 1998). Little is known about the blood-brain barrier function in human brain trauma and the contribution of cellular and extracellular oedema to post traumatic brain swelling and intracranial hypertension. It is reasonable to state that both types co-exist and should be considered in the care of patients. In our material we could show a pattern compatible with picture from animal studies, with early and sustained increase in small molecular permeability. The methodology was, however, a bit too cumbersome and time consuming to fit into clinical practice, where time often is limited. Recent upgrades at the department of radiology has made it possible to calculate K_i with commercially available software and with much shorter scan time. Perhaps in the future a “CT perfusion and permeability” can predict the risk of brain oedema development.

Conclusions

In animal models of brain trauma and meningitis, volume expansion with crystalloids promote brain oedema by diffusion into the interstitium and by favouring transvascular filtration by diluting the colloid osmotic pressure in plasma. Volume expansion with albumin infusions is safe with regard to oedema development and intracranial pressure in these experimental settings.

Experimental brain trauma induces a large increase in blood-brain barrier permeability to small solutes, whereas macromolecular permeability remains low enough for a sustained colloid osmotic gradient across the capillaries.

Infusion of prostacyclin in experimental meningitis counteracts systemic plasma volume losses and diminishes intracranial hypertension, probably because of reduced permeability or reduced capillary pressure.

Treatment with rosuvastatin after experimental brain trauma increases cortical capillary patency. The effect is associated with increased bioavailability of nitric oxide.

In human brain trauma, permeability changes of the blood-brain barrier in pericontusional tissue are detectable with the use of contrast-enhanced computerized tomography.

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Appendix

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