BRAIN INFLAMMATION AND CELL DEATH FOLLOWING INTRACEREBRAL HEMORRHAGE IN RODENTS

A Thesis Presented to the Faculty of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Department of Human Anatomy and Cell Sciences

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By

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LIST OF ABBREVIATIONS

AIF - Apoptosis induce factor

AVM - arterialvenous malformation

ANOVA - analysis of variance

BBB - blood brain barrier

BDNF - brain-derived neurotrophic factor

BCG - bacillus Calmette-Guerin

BSA – bovine serum albumin

CD - cluster of differentiation (standardized nomenclature for designation of leukocyte surface molecules)

CBF - cerebral blood flow

cm - centimeter

ConA - concanavalin A

CNS - central nervous system

CNTF - generation ciliary neurotrophic factor

CPP - cerebral perfusion pressure

CSF - cerebrospinal fluid

CTL - cytotoxic T lymphocyte

DNA - deoxyribonucleic acid

ELISA - enzyme-linked immunosorbent assay

EOM – enzyme overlay membrane

FGF - fibroblast growth factor

GFAP - glia fibrillary acidic protein

GM - germinal matrix

H&E - hematoxylin and eosin

HLA - human leukocyte antigen

ICAM - intracellular adhesion molecule

ICH - intracerebral hemorrhage

ICV - intracerebroventricular

ICP- intracranial pressure

IFN - interferon

IL - interleukin

iNOS - inducible nitric oxide synthase

ISNT - in situ nick translation

IVH - intraventricular hemorrhage

IUI - intrauterine infection

kg - kilogram

LPS - lipopolysaccharide

MAP - mean arterial pressure

MAPK - mitogen-activated protein kinase

MBP - myelin basic protein

MCA – middle cerebral artery

mg - milligram

MHC - major histocompatibility complex

MIF - macrophage/microglial inhibitory factor

ml - milliliter

mm - millimeter

MMPs - matrix metalloproteinases

MRI - magnetic resonance imageing

mRNA - messenger ribosonucleic acid

MS - multiple sclerosis

MTT - 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide]

NF-kB - nuclear factor kappa B

NK - natural killer lymphocytes

NMDA - N-methyl D-aspartate

NO - nitric oxide

PAI - plasminogen activator inhibitor

PAR - protease activated receptors

PBS - phosphate buffered saline

PDGF - platelet derived growth factor

PN-1 - protease nexin-1

PolyI:C - Polyinosinic-polycytidilic acid

PS - phosphatidylserine

PVH - periventricular hemorrhage

PVH/IVH - periventricular/intraventricular hemorrhage

RCA-1 - Ricinus communis agglutinin lectin

ROS - reactive oxygen species

SAH - subarachnoid hemorrhage

SCID - severe combined immune deficient

SEB - staphylococcal enterotoxin B

SHR - spontaneously hypertensive rats

TBI - trauma brain injury

TGF - transforming growth factor

TIMP - tissue inhibitors of metalloproteinases

TNF - tumor necrosis factor

tPA - tissue plasminogen activator

TUDCA - tauroursodeoxycholic acid

TUNEL - terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick end labeling

VCAM - vascular adhesion molecule

uPA - urokinase-type plasminogen activator

μg - microgram

μl - microliter

μm - micrometer

Chapter 1 GENERAL INTRODUCTION

1.1 Introduction and definitions

Stroke is defined as any acute clinical event, related to impairment of cerebral circulation, that lasts for more than 24 hours and can cause a loss of the ability to move particular parts of the body. The sudden death of some brain cells is due either to lack of oxygen when the blood supply to the part of the brain is suddenly interrupted (ischemic stroke) or to bursting of a blood vessel in the brain, which spills blood into the spaces surrounding the brain cells (hemorrhagic stroke, intracerebral hemorrhage, ICH). Subarachnoid hemorrhage (SAH) implies the presence of blood within the subarachnoid space from some pathologic process. The nontraumatic types of SAH are usually from rupture of a berry aneurysm or arteriovenous malformation (AVM). We will not discuss SAH because blood does not necessarily enter brain parenchyma. Brain cells are damaged when stroke happens. These damaged cells can linger for several hours in the penumbra area, which surrounds the damaged core and contains functionally impaired but still viable brain tissue supplied with blood from collateral vessels. This area may be transformed into infarction due to secondary neuronal damage induced by deleterious biochemical cascades, resulting in cytotoxic and excitotoxic effects. With timely treatment, these cells can be saved.

ICH differs from ischemic stroke in that blood enters the brain parenchyma and may extend into the ventricles (Figure 1-1a, see page 15). The amount of research concerning ICH is lacking compared to the work done on ischemic stroke. Delayed

clinical deterioration may occur after ICH but the mechanism is not fully understood. After ICH blood components (i.e. leukocytes, hemoglobin, thrombin, plasmin, complement, plasma, and fibrin degradation products) enter into the brain parenchyma. This is followed by an inflammatory response and brain cell death, which may involve enzyme activation, cytokine release, leukocyte migration, and brain tissue breakdown and repair. Some aspects of the brain damage following ICH are well documented, for example, disruption of tissue by the enlarging hematoma [1], reduction of blood flow in surrounding tissue [2], and destruction of cells by proteolytic enzymes including thrombin, plasmin, and matrix metalloproteinases (MMPs) [3, 4].

Although ICH is a disease of the brain, it can affect the entire body due to neurological deficit. The consequences following ICH depend on the area of the brain affected. The most common neurological deficit is weakness or paralysis of one side of the body with partial or complete loss of voluntary movement or sensation in a leg or arm. There can be speech problems, cognitive deficits, emotional difficulties, daily living problems, and pain. Numbness or tingling is very common. An ICH involving the brain stem can affect balance, vision, swallowing, breathing, and even consciousness.

1.2 Hemorrhagic stroke in the clinical setting

1.2.1 Brain hemorrhage in the adult brain

The worldwide annual incidence of primary (spontaneous) ICH is 12-35/100,000 population, which accounts for approximately 15% of cerebral strokes and is associated with a higher mortality rate [5, 6] than to brain ischemia. ICH is more common in men than

in women, particularly those older than 55 years of age [7] and of African American and/or Japanese origin [8]. A high prevalence of hypertension and alcohol use in the Japanese population may account for the incidence [9]. ICH associated with hypertension [10] remains the most common form of ICH. ICH may also be associated with coagulopathy [11, 12], cerebral amyloid angiopathy [10], brain tumors [13], vascular anomalies [14], brain trauma [1], or premature birth [15]. Stroke associated with ICH has a worse prognosis than ischemic stroke [16], the mortality rate at 30 days is 43-51% [17]; and recovery following ICH is poor; most surviving patients retain a considerable functional handicap related to the specific site of hemorrhage [18]. The most common sites of hypertensive ICH are the caudate / putamen (basal nuclei), thalamus, cerebellum, and pons (the locations may differ with other etiologies).

There is no proven effective treatment for ICH [19]. However, the clinical therapeutic strategies include medication and surgery. Drug therapy is the most common treatment for ICH. This includes prevention of ICH based on treating an individual's underlying risk factors, for example, control of hypertension. The blood glucose in diabetics is often quite high after a stroke; controlling the glucose level may minimize the size of a stroke [20]. Oxygen is given as needed. Surgery can be used to prevent ICH by repairing vascular damage or malformations in and around the brain or to treat acute ICH by evacuating the hematoma, but the surgical treatment is still controversial [21, 22] due to very few controlled randomized trials. Rehabilitation may help overcome disabilities that result from ICH damage [23]. Initial severity of disability, age, and duration of therapy best predict functional outcome after rehabilitation.

1.2.2 Hemorrhage in the immature brain

The developing brain lacks mechanical rigidity and is readily deformed during birth or following trauma. Fragile blood vessels within the brain are thereby easily ruptured. Hemorrhage can arise from the arterial, capillary, or venous systems. It may be due to intravascular hypertension, thrombosis, or breakdown of vessel integrity following infarction or physical trauma [24]. The location and type of hemorrhage are dictated at least in part by the stage of development [25]. Periventricular hemorrhage (PVH) refers to bleeding adjacent to the lateral ventricles. Intraventricular hemorrhage (IVH) refers to blood collections within the ventricles, which are usually extensions of ganglionic eminence hemorrhage. PVH and IVH frequently occur in combination (PVH/IVH). Blood can extend into the ventricles causing hydrocephalus, or it can cause secondary ischemic injury in the adjacent tissue [15]. Even a small hemorrhage may be associated with cognitive deficits [26]. Detectable PVH/IVH with extensive hemorrhage occurs in approximately half of the affected infants born <28 weeks while only one tenth of those affected at >35 weeks have severe bleedings [27]. The highest risk periods are within the first 3 hours after birth, 2 days, and around 10 days after birth [28]. The main risk factor for IVH is young gestational age, although maternal / fetal sepsis [27, 29-32], and delivery with excessive head distortion may contribute [25].

The desirable therapy is prevention of brain hemorrhage through the avoidance of preterm birth, chorioamnionitis, and improved understanding of the physiologic determinants of hemorrhage. Many medical treatments have been attempted to prevent initial or progressive IVH in preterm infants, but none appear to be effective [33, 34]. Digestion of intraventricular clots with proteolytic agents such as urokinase and tPA has

been attempted with minimal success although new trials are being designed [35, 36]. Subdural hemorrhage can be managed surgically if necessary.

1.3 Animal models of hemorrhagic stroke

To understand the pathogenesis of ICH and to evaluate preventive or therapeutic strategies, animal models of ICH have been developed and used. Experimental ICH models have been studied in several species including mouse [37], rat [38-42], rabbit [9, 43], cat [44], pig [45], and primate [46].

1.3.1 Microballoon insertion models

An acute expanding lesion model using a mechanical microballoon to simulate the space-occupying effect of ICH was developed by Sinar in the adult rat in 1987 (although this model lacks the effects of blood components) [47]. The microballoon system consisted of an embolization balloon mounted on a 20 gauge venous cannula using its own previously blunted guide. The microballoon was then inflated with saline in a syringe. Immediately following balloon inflation in the caudate nucleus of rats, there was a significant increase in intracranial pressure accompanied by a reduction in cerebral blood flow (CBF) in the ipsilateral frontal cortex and the ipsilateral caudate nucleus [2]. There was little change in intracranial pressure (ICP) with microballoons (25 microliters and 50 microliters in volume) equivalent in size to those lesions which occur with this disorder in man. With larger volumes (100 microliters) there is an increase in ICP which is associated with systemic effects on cerebral perfusion pressure (CPP) [48]. The rats with an intracerebral mass exhibited a 10-fold increase in the volume of ischemic damage in

the ipsilateral caudate nucleus compared to the sham-treated group [47]. The quantity of damaged neurons was significantly higher in the permanent groups than in transient inflation groups [49]. Deflation of balloon after 10 minutes was shown to improve clinical outcome and reduces CBF abnormalities in rats [50]. Therefore, in this model evacuation of an extensive acute expanding subcortical (hematoma-like) mass must be performed within a limited time window to prevent the development of irreversible neurological deficits or death. Similarly a microballoon inserted into the ventral posterolateral nucleus of the thalamus in cat, caused a rapid reduction in CBF following gradual balloon inflation [51].

1.3.2 Autologous whole blood injection models

Although autologous blood does not reproduce the rupturing of a blood vessel in spontaneous ICH, this method allows anatomically localized hematomas to be created without artificial agents [39-41]. The autologous whole blood may be obtained from the animal's tail or femoral artery, then directly injected into the selected brain areas. Sometimes the femoral artery may be attached directly to a cannula to simulate pressure/pulsation. Several groups have studied the brain injury mechanism following ICH by using this model. In the rat, autologous blood can cause brain edema, cell death, inflammation and behavioral impairment [38-40, 52-54]. Studies in the dog have shown that despite a prominent increase in intracranial pressure (ICP) and mean arterial pressure (MAP) after ICH, there is no evidence to support the presence of an ischemic penumbra in the first 5 hours after ICH [55]. Increased intracranial pressure as well as compromised CBF and metabolism following ICH have been shown [56] in the cats, rabbits, monkeys,

and pigs. Pigs have been frequently studied for clot evacuation [57, 58]. For instance, a tPA-induced clot lysis study showed that reduction in clot size was significantly greater than mechanical aspiration alone. In the rabbit model [59], urokinase treated animals showed 86 % of clot lysis compared to injection of saline into clot (23 %).

Rat models have been used to compare effects of blood components, such as thrombin, plasmin, plasma, serum, leukocyte fractions and erythrocytes individually [40-42]. Leukocytes, activated leukocytes, thrombin and plasminogen caused brain edema, inflammation, and brain cell death when they were injected into the brain [38]. Components of the coagulation system can modulate inflammation [60]. Activation of the complement system [61] and injections of hemoglobin as well as erythrocytes into the brain may lead to brain edema [41, 42].

1.3.3 Collagenase animal models of ICH

This model was developed by Rosenberg's group. Bacterial collagenases, which are proteolytic enzymes, are injected into basal ganglia to induce ICH by destruction of the capillary basal lamina in the brain [62, 63]. The advantage of using this model is that it produces highly reproducible hemorrhages and mimics spontaneous ICH without significant blood leakage along the needle track. In studies of adult rat following collagenase induced ICH, behavioral improvement is rapid during resolution of the edema but incomplete at 3 weeks [52]. This model is also used to study treatment following ICH [4, 62-65]. A disadvantage of using the collagenase model is that it involves introduction of a foreign protein that seems to cause more inflammatory reaction than autologous

blood injection or venous hemorrhage due to avulsion of surface vessels of brain [54]. Addition of heparin to collagenase injection enhances the inflammation in rat brain [52].

1.3.4 Hypertensive stroke models

Hypertension is the most common cause of primary ICH. Hypertension also causes changes in the walls of small vessels in the brain and leading to rupture, which make the blood bleed into the brain parenchyma. To understand the effect of hypertension induced hemorrhage and to develop treatment for it, several animal models have been developed [66]. Renal artery constriction in which the roots of both renal arteries are constricted by ring-shaped silver clips, causes renovascular hypertension [67]. The rate of stable hypertension was 100% and the incidence of spontaneous stroke including ICH and brain infarct was 61.8% at 40 weeks after renal artery constriction [67]. The hypertension is stable and not renin dependent, apparently involving brain angiotensin and perhaps circulating vasopressin [66]. Stroke prone spontaneously hypertensive rats (SHR) may also develop cerebral hemorrhage as well as cerebral infarct [68]. The brain lesions in this model include old and fresh cerebral hemorrhage and infarcts with or without subarachnoid effusion. These models simulate hypertensive ICH in humans and offer the chance to study the mechanism of brain injury following hypertensive ICH. The disadvantages are that brain lesions are unpredictable with regard to size and position.

1.3.5 Models of neonatal PVH/IVH and IVH

Using immature rabbits, dogs, cats, and sheep, the mechanisms of germinal matrix hemorrhage have been elucidated. Fluctuations in arterial and venous blood

pressure can cause PVH [69]. IVH has been induced using glycerol to create intracranial hypotension in prematurely born rabbits (27 - 30 days gestation) [70, 71]. In a newborn beagle model, injection of phenylephrine hydrochloride intravenously induced hypertension which can cause IVH [72]. Intraventricular injection of blood into newborn dog brains has been used to study the effect of acute ventricular distension on the surrounding blood flow patterns [73]. Dog models have played an important role in understanding the physiologic factors that predispose to PVH/IVH [69]. A mouse model of neonatal hypoxia develops superficial foci of bleeding unlike those seen in humans [74]. We recently developed a novel PVH/IVH model in newborn mice by injection of autologous whole blood into periventricular tissue including germinal matrix (GM) and striatum [37]. All mice exhibited extension of the hematoma into the ventricles, which mimics germinal matrix hemorrhage in humans at 24-28 weeks gestation age. This model provides an opportunity to study mechanisms of cellular injury after PVH/IVH. Posthemorrhagic hydrocephalus can be modeled by injection of blood into the ventricles of 7-day old rats [75].

1.3.6 Other animal models of ICH

In addition to the above-mentioned ICH animal models, other models have also been developed. Cortical vessel avulsion by tearing the pia can cause mixed brain damage including ischemic and hemorrhagic [54]. Hemorrhage related to shaking injury in 6-day old rats has been studied as a model of child abuse [76]. Some forms of traumatic brain injury (TBI) also cause bleeding into the brain parenchyma [77-79]. None of the above-mentioned ICH models completely reproduce the brain injury response following

human ICH. However, these models have significantly contributed to the overall knowledge of the pathophysiology of human ICH including edema, inflammation, cell death, brain damage, compromised CBF, and metabolism as well as pathogenesis.

1.4 Histopathology of ICH

Following ICH, blood components, including cells and plasma, intermingle with brain cells adjacent to the hematoma, which is a contiguous collection of clotted blood. Brain tissue surrounding the core of damaged area appears pale due to edema. After 24 h, degenerating erythrocytes and fragmented nuclear debris are seen. Hemosiderin is evident in macrophages as early as 3 days after the bleed [80]. The surrounding tissue may become necrotic if the hematoma is large and secondary infarction ensues [81]. In intact brain tissue surrounding the hematoma, neutrophils adhere to vessel walls or pass through capillaries and small veins. Neutrophils are rarely present within the necrotic tissue except at the periphery of hematoma. Neutrophil infiltration and reactive glial changes including astrocyte activation and microglia reaction in the brain adjacent to the hematoma are obvious at 2-3 days after ICH. Large clots degrade very slowly because the macrophage ingestion of debris takes place only at the surface. For months after clot resolution, residual hemosiderin and mineralization may be detected along the hematoma cavity. In the IVH, blood debris may obstruct the cerebral aqueduct. This may cause hydrocephalus.

1.5 Pathophysiology of brain damage after ICH

Several mechanisms are involved in ICH-related brain damage. Mechanical