SUPPLEMENTAL MATERIAL

Comparison of Large Animal Models for Acute Ischemic Stroke: which model to use?

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Circulating blood volume and blood sampling

Blood sampling is essential in experimental studies, and repeated sampling is often required to collect reliable longitudinal data sets associated with the lesion, therapeutic intervention, prognosis and/or recovery. An advantage of larger animals for focal ischemia studies derives from their larger blood volume as compared to rodents. This allows adequate sampling for i) examination of leukocyte and immune cell subsets, as well as other blood cells and plasma components, ii) pharmacodynamic and pharmacokinetic studies of specific interventions, iii) control of pharmacologic intervention effects in blinded studies, iv) studies involving CNS–peripheral blood interactions, and v) other exploratory (e.g. biomarker) studies without impact on the cardiovascular system of the model during the experiment. Blood sampling should not induce unnecessary stress to the animal as this may influence experimental results. The maximal volume of single samples depends on the recovery time but should never exceed 10% of the total blood volume to prevent hypovolemic shock. A withdrawal of 10% will require a recovery period of at least 2 weeks.[73] If daily samples are required, 1% of the total blood volume can be drawn daily up to two weeks, followed by a recovery period of at least two weeks. Table I summarizes the total blood volumes and maximal sample volumes for different species and strains. When considering biomarker studies that require repetitive blood sampling, maximal sampling volumes should be taken into account.

Coagulation and immune function

Table II summarizes coagulation values found in literature. Dogs, swine and NHP are reported to have platelet counts at the high end of human reference values, while sheep have higher platelet counts than humans. When specifically comparing swine and sheep platelet responses to biomaterials, sheep platelets attach less, do not fully spread, and are less active overall. Swine platelets react to biomaterials similar to human platelets.[74] Interestingly, sheep have clotting times similar to humans (sheep 494 [344-1431] versus human 595 [476-901] sec; median [range]), whereas clotting times in swine (244 [146-296] sec) appear shorter than in humans.[75] Conversely, the fibrinolytic system of swine more closely resembles that of humans.[75] More studies comparing coagulation tests of all large animals are needed, as inter-study comparison is difficult because of different laboratory techniques. Moreover, coagulation tests can be considerably influenced by sex- and strain-specific differences, as well as by nutrition.[76]

The pathophysiology and possible therapeutic modulation of the immune/inflammatory response after ischemia and reperfusion is of special interest. Brain ischemia is known to activate microglial cells, increase expression of cytokines and reactive oxygen species, and to stimulate influx of circulating inflammatory cells like neutrophils and lymphocytes.[77] Although the immune response is a well-conserved mechanism among mammals, peculiarities of large-animal species should be considered. An example is that γδ T cells, unconventional lymphocytes, represent up to 50% of the total blood lymphocyte population in young pigs but, conversely, this subpopulation of T cells is less than 10% in humans.[78] Sheep also have a greater proportion of circulating γδ T cells than humans.[79] Another characteristic of swine lymphocyte populations is the large presence of a unique subpopulation of CD4+ T cells that are also CD8α+, which is typically not observed in humans or other species.[78,80] In this sense, differences in lymphocyte subpopulations can also be observed in NHP (Rhesus macaque), such as the presence of two unique subsets of B cells in circulating blood that express CD1c or CD8α and that are rare in humans.[81] As comparative studies on the immune system of large animals are scarce, there is not yet enough information to base the selection of the animal model on this aspect.

Another important consideration is the influence of stress and health status on the immune function. Stress can alter both innate and adaptive immune responses and modify the leukocyte

distribution, cytokine secretion, lymphocyte proliferation or antibody production. For example, stress caused by weaning can increase the neutrophil/lymphocyte ratios in piglets,[82] and can affect the adult immune status.[83] Moreover, lymphoid tissue weights and percentages of immune cell subsets can be influenced by animal health status (i.e. farm or specific pathogen-free pigs).[80] Immune function of NHP can also be affected by social stress, as reorganizations of animal groups can alter lymphocyte levels and reactivity.[84] Although the current standards of animal welfare in biomedical research focusses on minimizing stress, possible influences of acute stressful situations on immune function, such as stress leukocytosis induced by temporary immobilization in non-trained animals, should always be considered for all species.

Table I. Recommendations for blood sampling volumes.

	Dog	Mini Swine	Swine	Sheep	Marmoset	*Macaca Mulatta*
Average weight in experiments (kg)	15	20	60	50	0.35	9
Total blood volume (mL)	1130	1300	3900	3300	25	480
Maximum daily sampling volume (mL)	11.3	13	39	33	0.25	4.8
Maximum sample volume e.g. during terminal experiment (mL)	113	130	390	330	2.5	48

Table II. Coagulation parameter values.

Coagulation values	Human	Dog	Swine	Sheep	NHP
Platelet count (x 10^9/mL)[85]	150-370	350	350	550	370*
Prothrombin time (s)[86]	10.9-13.3	7.4	11.4	16.4	12.0†
Partial thromboplastin time (s)[86]	22-32	17.7	16.6	36.1	36.8†
Thrombin time (s)[86]	13-26	15.3	22.6	13.1	20.3†
Fibrinogen (mg/ml)[86]	3.25	1.86	2.0	3.7	-
Plasminogen (U/mL)[86]	0.85-1.20	0.038	0.036	0.01	32†
Antithrombin (U/mL)[86]	0.80-1.20	1.24	1.01	-	1.32†

NHP indicates non-human primate; *Baboon; † Macaca fascicularis.

Table III. Contemporary approximate costs per animal in Europe, USA and China.

Cost per Animal	Dog	Swine	Sheep	*Macaca Mulatta*
Europe*	$1500-2500	$400-1500	$100-400	$12000-18000
USA	$1000-1500	$200-500	$200-500	$6000-12000
China**	$150-250	$450-500	$250-300	$1500

* 1.00 euro (€) = 1.20 US dollars ($); ** 1.00 yuan (¥) = 0.15 US dollars ($)

Table IV. Non-human primate stroke models

Methodology	Location	Species	Approach		Purpose
Clipping					
Crowell (1970)	prox M1	Macaca *sp*	TC	An	Neuropathology
Hudgins (1970)	prox M1	Saimiri *sp*	TO	An	Neuropathology
Symon (1975)	prox/dist M1	Papio *sp*	TO	An	Cerebral blood flow
Liu (1992)	common ACA	Papio *sp*	TO	An	Cerebral blood flow
Young (1997)	prox/dist M1	Papio *sp*	TO	An	Ischemia-reperfusion
Frazee (1998)	prox M1	Papio *sp*	TO	An	Retrograde perfusion
Huang (2000)	ICA/ACA	Papio *sp*	TO	An	Larger infarction
Extrinsic balloon compression					
Spetzler (1980)	prox M1	Papio *sp*	TO	Aw	Clinical
Del Zoppo (1986)	prox M1	Papio *sp*	TO	Aw	Multiple studies
Coagulation					
Yonas (1990)	LSA	Papio *sp*	TO	An	Striatal infarction
Nudo (1996)[87]	cortical branch	Saimiri *sp*	TC	An	Cortical infarction
Marshall (2003)[63]	M1	Callithrix *sp*	TC	An	MCA infarction
Snare ligation					
Crowell (1981)	prox M1	Macaca *sp*	TO	Aw	Time course studies
Embolization					
Molinari (1974)	M1	Macaca *sp*	IA	Aw	Endovascular approach
Watanabe (1977)	prox M1	Papio *sp*	IA	An	Endovascular approach
Endothelin-1					
Virley (2004)[88]	M2	Callithrix *sp*	TC	An	Cortical lesions

This table was adapted from S Fukuda and GL del Zoppo, *Ilar J*, 2003, to which we refer for references.[59] Nudo (1996), Marshall (2003) and Virley (2004) have been added.

Prox = proximal, dist = distal, M1 = first branch of middle cerebral artery, M2 = second branch of middle cerebral artery (distal to first bifurcation), ACA = anterior cerebral artery, ICA = internal carotid artery, LSA = lenticulostriate arteries, TC = transcranial, TO = transorbital, IA = intraarterial, Aw = awake, An = anesthetized.