EDITORIAL

Insights Into the Development and Treatment of Cardiovascular Disease: A Role for Animal Models

Approximately one-third of the total deaths worldwide per annum, amounting to 16.6 million people, is due to cardiovascular disease [1], making it the number one cause of death [2]. Cardiovascular disease also has a major impact on morbidity [1]. Thus, reducing the incidence of deaths due to vascular disease/complications and developing better treatment strategies, remains a central goal for national economies worldwide [3, 4]. Consequently, several animal models have been developed to replicate human vascular diseases, in order to study the pathophysiology of disease progression and novel therapeutic options. This issue of The Open Cardiovascular Medicine Journal discusses some of these models. In this Editorial, each review article for the Special Issue will be briefly outlined, with consideration given to a few additional models.

Tsui describes genetically predisposed, chemical and physical animal models of abdominal aortic aneurysms (AAAs) [5]. This review focuses on the pathophysiological mechanisms that underlie the development and progression of AAAs and the different treatment modalities for their management.

Ou *et al.* discusses the development of animal models of myocardial infraction, dilated cardiomyopathy, heart failure, myocarditis and cardiac hypertrophy, emphasising the usefulness of stem cell therapy [6].

Karasu describes abnormal cardiac contractility and impaired vascular reactivity in animal models of diabetes mellitus and highlights the role of antioxidant therapy in preventing or delaying diabetic cardiovascular complications [7].

Grossman explores the use of the renal hypertension-Goldblatt kidney and uraemic cardiomyopathy animal models to look at the relationship between cardiovascular disease and renal pathophysiology [8].

Ameen and Robson describe the relationship between Duchenne Muscular Dystrophy and cardiovascular disease in spontaneous and transgenic animal models, outlining new treatment options [9].

Price *et al*. discusses the use of magnetic resonance imaging to follow the pathophysiology of myocardial ischaemia and atherosclerosis in animal models. Attention has also been given to imaging the rodent developing heart to assess the influence of genes and congenital diseases [10].

In addition, several other models have been developed for the study of vascular disease. This Editorial briefly addresses some of them.

1. ISCHEMIC AND HEMORRHAGIC STROKE

Strokes result from ischemic (brain infarction), as well as, intracerebral or subarachnoidal hemorrhage [11]. Ischemic stroke is often caused by occlusion of the middle cerebral artery or one of its branches. The most relevant animal model has emerged from isolated middle cerebral artery occlusion in rodents. One of the striking features of this model is the ease to perform both permanent and transient ischemia in a controlled manner [12]. Thromboembolic stroke, the most common stroke type in humans, can be mimicked in rodents and used for preclinical testing of thrombolytic agents [13-15], evaluation of the ischemic lesion under the effect of thrombolysis [16, 17], investigation of the consequences of thrombolysis such as hemorrhagic transformation [18], to test novel antithrombotic agents [19, 20] and combination therapies (e.g. thrombolytic agents and neuroprotective drugs) [21-23].

Strokes caused by intracerebral and subarachnoid hemorrhages are associated with high mortality and most survivors are burdened with severe disability. Several animal models have been developed to study intracerebral haemorrhages [24-27]. The most commonly used models involve: autologous blood or collagenase injection [28-30] or balloon inflation [31] into the desired brain region.

Subarachnoid haemorrhages have also been studied in various animal models [32-35].

The endovascular filament rupture of the basilar artery and intracranial internal carotid artery bifurcation models have become very popular [30, 36, 37].

In general, animal stroke models are able to reproduce important pathophysiological events relevant to the human situation and help in the development of novel treatment regimes.

2. HYPERTENSION

Hypertension is an important risk factor for cardiovascular and cerebrovascular disease. The availability of animal models for research on the pathophysiology and treatment of hypertension-induced disease has provided valuable information. Models

1874-1924/10 2010 Bentham Open

have been created following portal vein stenosis [38], renal surgery [39], high fructose diet [40] and the administration of deoxycorticosterone acetate salt [41]. However, spontaneously hypertensive rats represent the most common animal model [42-46]. Cerebrovascular changes, brain atrophy, loss of nerve cells in cerebrocortical areas is evident in these rats; allowing the assessment of the effectiveness of anti-hypertensive therapy on these changes.

3. PERIPHERAL ARTERIAL DISEASE

Animal models for hindlimb ischemia are useful for investigating many of the features of peripheral arterial disease (PAD) [47, 48], such as angiogenesis and arteriogenesis [49]. They have been used to evaluate the beneficial effect of autologous bone marrow cell infusion [50, 51], vascular endothelial growth factor [52] and platelet-derived endothelial cell growth factor [53] for the induction of angiogenesis. In addition, animal models have helped establish diagnostic tests for the evaluation and quantification of angiogenesis [54-57].

These models provide insight into the pathophysiology and management of PAD. Application of these preliminary results in humans holds implications for a different therapeutic approach to this disease in the future.

4. ERECTILE DYSFUNCTION

The association between erectile dysfunction (ED) and coronary heart disease has confirmed that it is another manifestation of atherosclerotic vascular disease. Interestingly, the same risk factors (e.g. diabetes mellitus, hypertension, dyslipidaemia, and smoking) predict both ED and vascular disease [58, 59]. These risk factors and their association with ED have been investigated in many animal studies.

The disruption of the nitric oxide (NO)/ cyclic guanosine monophosphate (cGMP) pathway seems to be a unifying factor in many of these conditions, resulting in a reduction in NO bioavailability and impaired corpus cavernosal smooth muscle relaxation, a cardinal component of the erectile process. The preservation of cGMP and the subsequent increase in smooth muscle relaxation, following treatment with phosphodiesterase type-5 (PDE5) inhibitors, represent an effective mode of treating diabetic ED [60]. One such drug, DA-8159 was found to ameliorate the development of ED in diabetic rats [61, 62].

Oxidative stress (OS) is also a feature of diabetes mellitus and ED, it is defined as an increase in the steady-state levels of reactive oxygen species, including superoxide anions (O_2^-), which occurs as a result of increased free radical generation and/or decreased anti-oxidant defense mechanisms. Not surprisingly antioxidant therapy has been pursued as a treatment option for diabetic ED. Low dose treatment with alpha-lipoic acid (antioxidant) and gamma-linolenic acid (omega-6-essential fatty acid) interacted synergistically to improve NO-mediated corpus cavernosal relaxation in diabetic rats [63]. While the antioxidant vitamin E enhanced the therapeutic effect of PDE5 inhibition, due to a reduction in OS in diabetic rats [64]. *In vivo* adenoviral gene transfer of EC-superoxide dismutase (SOD) reduced corporal O_2^- levels and raise cavernosal cGMP levels by increasing NO bioavailability thus restoring erectile function in diabetic rats [65].

Many animal models of hypertension have revealed the close association between hypertension and ED. An increase in OS has been implicated in this relationship. For example, rats infused with angiotensin II developed hypertension and ED, due to an increase in NADPH activity (an inducible source of O_2^- production). Apocynin an inhibitor of NADPH was found to exert protective effects on erectile function in this model [66]. Antioxidant treatment with alpha-tocopherol was found to improve erectile function in spontaneously hypertensive rats by increasing SOD activity, which reduced O_2^- levels [67]. While, PDE5 inhibition with angiotensin II receptor blockade improved the function and morphology of erectile tissue taken from spontaneously hypertensive rats [68].

Rabbit models have been successfully used to demonstrate the link between hypercholesterolaemia and ED. A conscious rabbit model has been developed to assess the potential that intravenously administered drugs have for treating ED [69,70]. For example, the impaired erectile response exhibited by hypercholesterolaemic rabbits was improved following PDE5 inhibition [70]. This class of drugs was also effective in treating hypercholesterolaemic rats with ED [71].

The development of transgenic animal models, in particular the apolipoprotein E knockout mouse has provided a suitable model to illustrate atherosclerosis-associated ED and to develop new therapeutic strategies targeted at both atherosclerosis and ED [72, 73].

Evidence based analysis of the role of smoking in the development of ED, suggests that they are linked [74]. This is supported by a recent study using mice; animals that received short-term exposure to secondhand smoke were found to develop ED due to an increase in OS, which was improved by PDE5 inhibition [75].

It is clear that animal models play a pivotal role in the study of the pathophysiology of cardiovascular disease. The development of new models in the future will undoubtedly increase our understanding of the cellular/ molecular events involved in disease progression and aid the development of novel treatment strategies.

ABBREVIATIONS

AAA = Abdominal aortic aneurysms

cGMP = Cyclic guanosine monophosphate

DM = Diabetes mellitus

ED = Erectile dysfunction

NADPH = Nicotinamide adenine dinucleotide phosphate

NO = Nitric oxide

PAD = Peripheral arterial disease

OS = Oxidative stress

PDE5 = Phosphodiesterase type 5

SOD = Superoxide dismutase

O_2^- = Superoxide anions

REFERENCES

[1] Poole-Wilson P. The prevention of cardiovascular disease worldwide: whose task and WHO's task. Clin Med 2005; 5: 379-84.
[2] World Health Organization. In Fact sheet No 317 Feb 2007 (World health organization, 2007) (http://www.whoint/mediacentre/factsheet/fs317/en/index.html).
[3] Minino AM, Heron MP, Murphy SL, Kochanek KD. Deaths: final data for 2004. Natl Vital Stat Rep 2007; 55: 1-119.
[4] Wong NC. Coronary artery disease – eighth international congress. From prevention to intervention. I Drugs 2009; 12: 742-46.
[5] Tsui J. Experimental models of abdominal aortic aneurysms. Open Cardiovasc Med J 2010; 4: 221-30.
[6] Lailiang O, Wenzhong L, Yi L, *et al*. Animal models of cardiac disease and stem cell therapy. Open Cardiovasc Med J 2010; 4: 231-39.
[7] Karasu C. Glycoxidative stress and cardiovascular complications in experimentally-induced diabetes mellitus: effects of antioxidant treatment. Open Cardiovasc Med J 2010; 4: 240-56.
[8] Grossman R. Experimental models of renal disease and the cardiovascular system. Open Cardiovasc Med J 2010; 4: 257-64.
[9] Ameen V. Robson LG. Experimental models of Duchenne Muscular Dystrophy: relationship with cardiovascular disease. Open Cardiovasc Med J 2010; 4: 265-77.
[10] Anthony NP, King KC, Jon OC, *et al.* Cardiovascular magnetic resonance imaging in experimental models. Open Cardiovasc Med J 2010; 4: 278-92.
[11] Feigin VL, Lawes CM, Bennett DA, Anderson CS. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. Lancet Neurol 2003; 2: 43-53.
[12] Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989; 20: 84-91.
[13] Papadopoulos SM, Chandler WF, Salamat MS, Topol EJ, Sackellares JC. Recombinant human tissue-type plasminogen activator therapy in acute thromboembolic stroke. J Neurosurg 1987; 67: 394-8.
[14] Overgaard K. Thrombolytic therapy in experimental embolic stroke. Cerebrovasc Brain Metab Rev 1994; 6: 257-86.
[15] Sakurama T, Kitamura R, Kaneko M. Tissue-type plasminogen activator improves neurological functions in a rat model of thromboembolic stroke. Stroke 1994; 25: 451-6.
[16] Brinker G, Franke C, Hoehn M, Uhlenkuken U, Hossmann KA. Thrombolysis of cerebral clot embolism in rat: effect of treatment delay. Neuroreport 1999; 10: 3269-72.
[17] Takano K, Carano RA, Tatlisumak T, *et al*. Efficacy of intra-arterial and intravenous prourokinase in an embolic stroke model evaluated by diffusion-perfusion magnetic resonance imaging. Neurology 1998; 50: 870-5.
[18] Tejima E, Katayama Y, Suzuki Y, Kano T, Lo EH. Hemorrhagic transformation after fibrinolysis with tissue plasminogen activator: evaluation of role of hypertension with rat thromboembolic stroke model. Stroke 2001; 32: 1336-40.
[19] Wang X, Xu L, Wang H, *et al*. Inhibition of factor Xa reduces ischemic brain damage after thromboembolic stroke in rats. Stroke 2003; 34: 468-74.
[20] Toomey JR, Valocik RE, Koster PF, *et al.* Inhibition of factor IX(a) is protective in a rat model of thromboembolic stroke. Stroke. 2002; 33: 578-85.
[21] Sereghy T, Overgaard K, Boysen G. Neuroprotection by excitatory amino acid antagonist augments the benefit of thrombolysis in embolic stroke in rats. Stroke 1993; 24: 1702-8.
[22] Zhang L, Zhang ZG, Zhang C, Zhang RL, Chopp M. Intravenous administration of a GPIIb/IIIa receptor antagonist extends the therapeutic window of intra-arterial tenecteplase-tissue plasminogen activator in a rat stroke model. Stroke 2004; 35: 2890-5.
[23] Romanos E, Planas AM, Amaro S, Chamorro A. Uric acid reduces brain damage and improves the benefits of rt-PA in a rat model of thromboembolic stroke. J Cereb Blood Flow Metab 2007; 27: 14-20.
[24] Clark W, Gunion-Rinker L, Lessov N, Hazel K. Citicoline treatment for experimental intracerebral hemorrhage in mice. Stroke 1998; 29: 2136-40.
[25] Kaufman HH, Pruessner JL, Bernstein DP, Borit A, Ostrow PT, Cahall DL. A rabbit model of intracerebral hematoma. Acta Neuropathol 1985; 65: 318-21.
[26] Kobari M, Gotoh F, Tomita M, *et al*. Bilateral hemispheric reduction of cerebral blood volume and blood flow immediately after experimental cerebral hemorrhage in cats. Stroke 1988; 19: 991-6.
[27] Mun-Bryce S, Wilkerson AC, Papuashvili N, Okada YC. Recurring episodes of spreading depression are spontaneously elicited by an intracerebral hemorrhage in the swine. Brain Res 2001; 888: 248-55.
[28] Xue M, del Bigio MR. Intracortical hemorrhage injury in rats: relationship between blood fractions and cell death. Stroke 2000; 31: 1721-7.
[29] Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT. Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. J Neurosurg 1994; 81: 93-102.
[30] Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. Stroke 1990; 21: 801-7.

[31] Lopez Valdes E, Hernandez Lain A, Calandre L, Grau M, Cabello A, Gomez-Escalonilla C. Time window for clinical effectiveness of mass evacuation in a rat balloon model mimicking an intraparenchymatous hematoma. J Neurol Sci 2000; 174: 40-6.
[32] Barry KJ, Gogjian MA, Stein BM. Small animal model for investigation of subarachnoid hemorrhage and cerebral vasospasm. Stroke 1979; 10: 538-41.
[33] Johshita H, Kassell NF, Sasaki T. Blood-brain barrier disturbance following subarachnoid hemorrhage in rabbits. Stroke 1990; 21: 1051-8.
[34] Lougheed WM, Tom M. A method of introducing blood into the subarachnoid space in the region of the circle of Wills in dogs. J Neurosurg 1961; 4: 329-37.
[35] Dorsch N, Branston NM, Symon L, Jakubowski J. Intracranial pressure changes following primate subarachnoid haemorrhage. Neurol Res 1989; 11: 201-4.
[36] Veelken JA, Laing RJ, Jakubowski J. The Sheffield model of subarachnoid hemorrhage in rats. Stroke 1995; 26: 1279-84.
[37] Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke1989; 20: 84-91.
[38] Albornoz L, de las Heras M, Bildozola M, bandi JC, Mastai RC. Chronic administration of polylthiouracil ameliorates hyperdynamic circulation in portal hypertensive rats. Gastroenterol Hepatol 2005; 28: 537-40.
[39] Mathur S, Brown CA, Dietrich UM, *et al*. Evaluation of a technique of inducing hypertensive renal insufficiency in cats. Am J Vet Res 2004; 65: 1006-13.
[40] Cossenzi A, Bernocbich E, Plazzotta N, Seculin P, Odoni G, Bellini G. Lacidipine reduces high blood pressure and the target organ damage induced by high fructose diet in rats. J Hypertens 1999; 17: 965-71.
[41] Elmarakby AA, Quigley JE, Imig JD, Pollock JS, Pollock DM. TNF-α inhibition reduces renal injury in DOCA-salt hypertensive rats. Am J Physiol Regul Interg Comp Physiol 2008; 294: R76-83.
[42] Sabbatini M, Tomassoni D, Amenta F. Influence of treatment with Ca(2+) antagonists on cerebral vasculature of spontaneously hypertensive rats. Mech Ageing Dev 2001; 122: 795-809.
[43] Sabbatini M, Tomassoni D, Amenta F. Hypertensive brain damage: comparative evaluation of protective effect of treatment with dihydropyridine derivatives in spontaneously hypertensive rats. Mech Ageing Dev 2001; 122: 2085-105.
[44] Blezer E, Nicolay K, Goldschmeding R, Koomans H, Joles J. Reduction of cerebral injury in stroke-prone spontaneously hypertensive rats by amlodipine. Eur J Pharmacol 2002; 444: 75-81.
[45] Amenta F, Di Tullio MA, Tomassoni D. Arterial hypertension and brain damage-evidence from animal models (review). Clin Exp Hypertens 2003; 25: 359-80.
[46] Amenta F, Tomassoni D. Treatment with nicardipine protects brain in an animal model of hypertension-induced damage. Clin Exp Hypertens 2004; 26: 351-61.
[47] Bitto A, Polito F, Altavilla D, Minutoli L, Migliorato A, Squadrito F. Polydeoxyribonucleotide (PDRN) restores blood flow in an experimental model of peripheral artery occlusive disease. J Vasc Surg 2008; 48: 1292-300.
[48] Li L, Okada H, Takemura G, *et al*. Sustained release of erythropoietin using biodegradable gelatin hydrogel microspheres persistently improves lower leg ischemia. J Am Coll Cardiol 2009; 53: 2378-88.
[49] Limbourg A, Korff T, Napp LC, Schaper W, Drexler H, Limbourg FP. Evaluation of postnatal arteriogenesis and angiogenesis in a mouse model of hind-limb ischemia. Nat Protoc 2009; 4: 1737-46.
[50] de Nigris F, Williams-Ignarro S, Sica V, *et al*. Therapeutic effects of concurrent autologous bone marrow cell infusion and metabolic intervention in ischemia-induced angiogenesis in the hypercholesterolemic mouse hindlimb. Int J Cardiol 2007; 117: 238-43.
[51] He Y, Luo Y, Tang S, *et al*. Critical function of Bmx/Etk in ischemia-mediated arteriogenesis and angiogenesis. J Clin Invest 2006; 116: 2344-55.
[52] Greve JM, Chico TJ, Goldman H, *et al*. Magnetic resonance angiography reveals therapeutic enlargement of collateral vessels induced by VEGF in a murine model of peripheral arterial disease. J Magn Reson Imaging 2006; 24: 1124-32.
[53] Yamada N, Li W, Ihaya A, *et al*. Platelet-derived endothelial cell growth factor gene therapy for limb ischemia. J Vasc Surg 2006; 44:1322-8.
[54] Sampath S, Raval AN, Lederman RJ, McVeigh ER. High-resolution 3D arteriography of chronic total peripheral occlusions using a T1-W turbo spin-echo sequence with inner-volume imaging. Magn Reson Med 2007; 57: 40-9.
[55] Dobrucki LW, Sinusas AJ. Imaging angiogenesis. Curr Opin Biotechnol 2007; 18: 90-6.
[56] Alnaeb ME, Alobaid N, Seifalian AM, Mikhailidis DP, Hamilton G. Optical techniques in the assessment of peripheral arterial disease. Curr Vasc Pharmacol 2007; 5: 53-9.
[57] Penuelas I, Aranguren XL, Abizanda G, *et al*. (13)N-ammonia PET as a measurement of hindlimb perfusion in a mouse model of peripheral artery occlusive disease. J Nucl Med 2007; 48: 1216-23.
[58] Sulivan ME, Thompson CS, Dashwood MR, *et al*. Nitric oxide and penile erection: Is erectile dysfunction another manifestation of vascular disease? Cardiovasc Res 1999; 43: 658-65.
[59] Jackson G, Rosen RC, Kloner RA, Kostis JB. The second Princeton consensus on sexual dysfunction and cardiac risk: new guidelines for sexual medicine. J Sex Med 2006; 3: 28-36.
[60] Supuran CT, Mastrolorenzo A, Barbaro G, Scozzafava A. Phosphodiesterase 5 inhibitors-drug design and differentiation based on selectivity, pharmacokinetic and efficacy profiles. Curr Pharm Des 2006; 12: 3459-65.
[61] Ahn GJ, Sohn YS, Kang KK, *et al*. The effect of PDE5 inhibition on the erectile function in Streptozotocin-induced diabetic rats. Int J Impot Res 2005; 17: 134-41.
[62] Ahn GJ, Yu JY, Choi SM, *et al*. Chronic administration of phosphodiesterase 5 inhibitor improves erectile and endothelial function in a rat model of diabetes. Int J Androl 2005; 28: 260-6.
[63] Keegan A, Cotter MA, Cameron NE. Corpus cavernosum dysfunction in diabetic rats: effect of combined alpha-lipoic acid and gamma-linolenic acid treatment. Diabetes Metab Res Rev 2001; 17: 380-6.
[64] De Young L, Yu D, Bateman RM, Brock GB. Oxidative stress and antioxidant therapy: their impact in diabetes-associated erectile dysfunction. J Andrology 2004; 25: 830-6.
[65] Bivalacqua TJ, Usta MF, Kendirci M, *et al*. Superoxide anion production in the rat penis impairs erectile function in diabetes: influence of *in vivo* extracellular superoxide dismutase gene therapy. J Sex Med. 2005; 2: 187-97.
[66] Jin L, Lagoda G, Leite R, Webb RC, Burnett AL. NADPH oxidase activation: a mechanism of hypertension-associated erectile dysfunction. J Sex Med 2008; 5: 544-51.
[67] Ushiyama M, Kuramochi T, Yagi S, Katayama S. Antioxidant treatment with alpha-tocopherol improves erectile function in hypertensive rats. Hypertens Res 2008; 31: 1007-13.
[68] Tobili JE, Cao G, Lombrana A, Rivero M. Functional and morphological improvements in erectile tissue of hypertensive rats by long-term combined therapy with phosphodiesterase type 5 inhibitor and losartan. J Sex Med 2007; 4: 1291-303.
[69] Bischoff E, Schneider K. A conscious-rabbit model to study vardenafil hydrochloride and other agents that influence penile erection. In J Impot Res 2001; 13: 230-35.

[70] Firoozi F, Longhurst PA, White MD. *In vivo* and *in vitro* responses of corpus cavernosum to phosphodiesterase-5 inhibition in the hypercholesterolaemic rabbit. BJU Int 2005; 96: 164-168.
[71] Kang KK, Yu JY, Yoo M, Kwon JW. The effect of DA-8159, a novel PDE5 inhibitor, on erectile function in the rat model of hypercholesterolemic erectile function. Int J Impot Res 2005; 17: 409-16.
[72] Xie D, Odronic SI, Wu F, Pippen AM, Donatucci CF, Annex BH. A mouse model of hypercholesterolemia-induced erectile dysfunction. J Sex Med 2007; 4: 898-907.
[73] Behr-Roussel D, Darblade B, Oudot A, *et al*. Erectile dysfunction in hypercholersterolemic atherosclerotic apolipoprotein E knockout mice. J Sex Med. 2006; 3: 596-603.
[74] MacVary KT, Carrier S, Wessells H. Smoking and erectile dysfunction:evidence based analysis. J Urol 2001; 166: 1624-32.
[75] Bivalaacqua TJ, Sussan TE, Gebska MA, *et al*. Sildenafil inhibits superoxide formation and prevents endothelial dysfunction in a mouse model of secondhand smoke induced erectile dysfunction. J Urol 2009; 181: 899-906.

Cecil S. Thompson
(Guest Editor)
Departments of Clinical Biochemistry & Surgery
Division of Surgery and Interventional Science
University College London Medical school
Royal Free Campus, NW3 2QG
UK
Tel: + 44 (0)2077940500
Fax: +44(0)2078302235
E-mail: cecil.thompson@nhs.net