

Animal Models for the Evaluation of the Cardiometabolic Therapeutic Potential of Traditional Chinese and Natural Product Medicines

Review Article

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Abstract

There is considerable interest in the potential for Traditional Chinese Medicines and other natural products to be used directly or as a source of small chemical entities for the treatment of diabetes and its associated cardiovascular disease. Thus, what is required is animal models which reproduce the pathophysiology of diabetes and cardiovascular with high fidelity and which thus can be used to test or screen natural products. Such a comprehensive model does not exist in a single animal model. Furthermore, the pathophysiology of diabetes is more complex than can be reproduced comprehensively in an animal model and with respect to cardiovascular disease the pathology of disease in animal models does not reflect the development of atherosclerotic plaques in human arteries. This review addresses these issues and suggests that different animal models are required to separately address effects on hyperglycaemia and atherosclerosis. It is further addressed that the therapeutic environment for the development of medicines for the treatment of diabetes and cardiovascular disease has changed considerably in the last year. Successful clinical trials have demonstrated the benefits in terms of reduced cardiovascular events and reduced deaths from the use of sodium glucose cotransport 2 inhibitors and glucagon like peptide agonists and this was achieved with a reasonable degree of safety. These results set a new benchmark for the development of new drugs in this area. A sophisticated approach to the evaluation of natural products for the treatment of cardiometabolic disease will expedite the discovery and development of new medicines in an area that has an exploding global population of people with diabetes.

Keywords: Diabetes; Atherosclerosis; GLP-1 Agonists; SGLT 2 Inhibitors; Hyperglycaemia; Type 2 Diabetes, Proteoglycans, Inflammation.

Introduction

There is a large amount of interest in the areas of Traditional Chinese Medicines (TCMs), underpinned by the rising influence of China in the world, and other natural product medicines [1] and similarly interest has risen in the area of diabetes, mostly Type 2 diabetes, where in the latter case the number of people affected has moved into the multiple hundreds of millions in the last few years [2]. This conjunction of events has led to a large increase in the interest in investigations of the potential role of TCMs as a source of new therapeutic agents for the treatment of diabetes and its associated cardiovascular disease [3, 4].

Diabetes is a complicated metabolic condition and it has

severe deleterious cardiovascular consequences, principally the accelerated atherosclerosis that leads to clinical cardiovascular events including death and disability from heart attacks and strokes [5, 6]. The spectrum of pathophysiologies that constitute diabetes includes beta cells status, insulin resistance and the status of the gut-based incretin system and these parameters are the first issue that needs to be addressed in evaluating any potential new therapeutic product for the treatment of diabetes. Thus, in evaluating TCMs and natural products, these elements need to be taken into consideration in choosing and utilising an animal model. There is not a high quality animal model that reflects these changes with high fidelity. Therefore, one or more models must be used which reliably evaluate the TCM or natural product medicine and provide an understanding of its potential as a

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therapeutic agent either directly or possibly as a source of new chemical entities.

The second major area is the consequences of diabetes to human health – if diabetes in the manner of hypertension or even smoking – did not have any adverse effects, then it would not be a health issue. However diabetes has such devastating and wide-spread effects on the cardiovascular system that it is possible to build a case that diabetes is a cardiovascular rather than metabolic disease [7, 8]. In this context it is not sufficient that any agent has an effect on hyperglycaemia, it must also have an impact on the accelerated atherosclerosis that is the response to the diabetic milieu. It needs to be considered and evaluated if the effect of an intervention to reduce cardiovascular disease is due to the reduction of the hyperglycaemia *per se* or a direct effect of the treatment or a mixture of both mechanisms. This can be done by using a suitable positive control with a known mechanism of action or by giving low dose insulin to reduce hyperglycaemia where it is known that this does not have an inhibitory effect on atherosclerosis.

Unfortunately, with the possible exception of primate models which are expensive and culturally sensitive, it has not been possible to develop an animal model that reliably reproduces the accelerated atherosclerosis as occurs in diabetes. Therefore it is necessary to address the issue of the effect of interventions on atherosclerosis independently, albeit that this may not be an ideal model for atherosclerosis in the setting of diabetes.

In relation to the discovery and potential development of any new therapies for diabetes, the prevailing therapeutic environment needs to be considered. Twenty years ago there were only 2 or 3 classes of oral diabetes drugs (sulfonylureas, biguanides) as the pharmaceutical industry world wide had been focusing on the development of medications for hypertension. Since industry and academic researchers appreciated the rapid rise in the diabetes as a major issue for human health, which commenced about 1980, they intensified research activity to develop new drugs for diabetes. The outcome is that there are more than 20 different classes of agents in clinical use and under development for the treatment of diabetes. Some of these new agents, specially sodium glucose co-transporter 2 (SGLT-2) inhibitors [9] and incretin mimetics [10, 11], have proven to be quite safe and efficacious in reducing adverse cardiovascular events and even deaths in people with Type 2 diabetes.

As well as efficacy, patient safety is a very important element of modern drug therapy. There is a notion that natural medicines are safe and Western medicines are toxic. However, Western medicines are subjected to intense post marketing surveillance to a level well beyond any such focus on the safety of natural product derived medicines. So studies of TCM and natural product medicines for the treatment of diabetes must offer a new, safer product and it must be identified as to where they would fit into the future therapy of diabetes specifically what is the mechanism of action.

Thus, to meaningfully, evaluate a TCM or alternative medicine, and indeed any new proposed therapy, it is desirable to investigate the effects on both the parameters of the metabolic disturbances in one of the available animal models and also to examine the effects on an animal model of atherosclerosis. In this article we will suggest two animal models – one based on hyperglycaemia

and insulin resistance and one for atherosclerosis – where an evaluation of the effects of a TCM or any other drug intervention would provide a basis for projecting that such an agent might be worthy of further detailed study as a potential therapeutic agent in humans.

Aetiology of Human Type 1 and Type 2 Diabetes

Very briefly, the human disease of Type 1 diabetes occurs due to immunological toxicity to pancreatic beta cells leading to destruction of beta cells and the failure of the cells to produce insulin in response to raised plasma glucose [12, 13]. Type 1 diabetes usually occurs in very young people but the age of onset has been rising and it may occur in adults even older adults. Patients with Type 1 diabetes have little or no endogenous insulin and for survival they must inject insulin daily for life. Insulin resistance is usually normal in people with Type 1 diabetes but this is a complicated and unresolved matter [14, 15].

The majority of cases of Type 2 diabetes arise secondarily to increasing obesity and rising insulin resistance over many years [16, 17]. This leads to rising blood glucose and rising insulin in what is termed a compensated phase (enough insulin can be produced to maintain normoglycemia). Simultaneously, where there are changes in the incretin system – the increasingly important and acknowledged area of gut hormones which favours the action of insulin and protects beta cells along with other metabolically favourable actions; the activity of the incretin system is reduced in Type 2 diabetes [18]. With constant high glucose and insulin, a phenomena known as lipoglucotoxicity is hypothesized to cause toxicity to beta cells, a destruction of beta cells and a failure to produce insulin in response to high blood glucose [6]. At this stage high blood glucose occurs in the presence of high blood insulin in a de compensatory phase; this is associated with wide spread metabolic abnormalities (e.g. low HDL cholesterol, small dense LDL particles) which is a potent environment for the rapid progression of atherosclerosis. The metabolic milieu of Type 2 diabetes also sensitizes the body to the pro-atherosclerotic effects of other cardiovascular disease risk factors such as hypertension leading to an additional impact on the development of atherosclerosis [6]. In the current context it is not easy to replicate these changes in animal models to assess the effects of TCM and natural products in a manner that relates to the human condition of diabetes.

In contrast to above aetiology, there is an appreciable group of people who develop high blood glucose without severe insulin resistance – these are thin people with Type 2 diabetes [19]. In this situation, pancreatic beta cell toxicity occurs probably (bio) chemically, in contrast to the immunologically induced toxicity of Type 1 diabetes but these patients do not have auto-antibodies associated with Type 1 diabetes. This population may represent about 20 per cent of the population of Type 2 diabetes patients. So one must refer specifically to the type of diabetes to which one is targeting and also in considering therapies what are the relevant specific therapies and what are the metabolic targets of these therapies. All of this needs to be taken into consideration when evaluating the action of TCM and natural product therapies on diabetes and cardiovascular disease.

An Animal Model for the Study of the Effects

of Drugs on Disturbed Metabolic Parameters of Diabetes

Although hyperglycaemia is the parameter used for the diagnosis of all types of diabetes, diabetes and hyperglycaemia are not the same thing – diabetes is a human disease condition with various metabolic disturbances one of which is hyperglycaemia [20, 21]. Treating a healthy young rat or mouse with a pancreatic beta cell toxin such as streptozotocin (STZ) destroys some pancreatic beta cells, reduces beta cell mass and function and generates an animal with hyperglycaemia [22-24]. To study the effect of a proposed “anti-diabetic” compound in such a model is really a toxicology study testing the effect of the intervention on the toxicity of STZ. The toxicity of STZ does not mimic the immunological toxicity of Type 1 diabetes and has only some factors (oxidative stress) in common with beta cell toxicity that occurs in Type 2 diabetes patients.

To mimic the substantial metabolic defect and condition in Type 2 diabetes, one needs to have a model which has both hyperglycaemia but also hyperinsulinemia (occurring due to insulin resistance as described above). Indeed an action to reduce plasma insulin is just as important and relevant as an action to reduce plasma glucose [24, 25].

An experimentally induced animal model of Type 2 diabetes is the high fat diet/STZ treated rat [24, 25]. This model involves a combination of a diet high in fat and/or cholesterol and in some cases with added sugar to bring about hyperinsulinemia, insulin resistance and glucose intolerance followed by the treatment with the beta cell toxin STZ which result in a severe reduction in functional beta cell mass. Together these two stressors may mimic the pathology of Type-2 diabetes in human condition [25, 26]. It should be noted that there are many other models of Type 2 diabetes which are variously suitable for such studies and these have been reviewed in detail [26, 27].

One under researched area is that of the incretin system in animal models of diabetes. So the importance of this system has risen very substantially in the last decade but its role in animal models follows behind. It is necessary to be aware of the prominent role of this system whilst considering the actions of TCM and other natural product derived therapeutic strategies.

An Animal Model for the Study of the Effects of Drugs on the Parameters of Atherosclerosis

In considering an animal model of atherosclerosis for the study of TCM or any intervention, it must be carefully noted that the development and progression of atherosclerosis in existing animal models is not the same as human atherosclerosis. Vermani and her colleagues [28] having analyzed in depth and detail the issue of atherosclerotic plaque formation and development in animals and humans concluded with the statement “... the mechanism of disease initiation and progression are quite different between mice and humans, with the former being a macrophage-rich disease and the latter occurring in a smooth-muscle-cell-rich environment with an extracellular matrix rich in proteoglycans, collagen, and entrapped lipids with sheets of calcification”. Atherosclerosis commences with the trapping of lipids in the vessel wall by modified proteoglycans with hyperelongated and “sticky”

glycosaminoglycan chains [29-33] and progresses into the long slow inflammatory phase involving macrophage infiltration, foam cell formation, plaque development and finally plaque rupture [34-36]. It has been exceedingly difficult to develop an animal model which mimics the development of human atherosclerosis and similarly very difficult to develop animal models that can reflect either the phenomena of plaque rupture or of the accelerated atherosclerosis occurring in the presence of the diabetic milieu. So animal studies have a bias towards the inflammatory phase of atherosclerosis and they need to be interpreted carefully in terms of human atherosclerosis. Furthermore, insulin resistance *per se* (i.e. aside from hyperglycaemia) is a risk factor for and driver of the atherosclerotic process [6]. Models which achieve hyperglycaemia without insulin resistance such as STZ treated animals represent an investigation of just one element, hyperglycaemia, and are not studies of the impact of the metabolic milieu in Type 2 diabetes on the progression of atherosclerosis [20].

In contrast to humans, animals including rodents, do not develop atherosclerosis spontaneously even when fed high fat diets for a prolonged period. Thus, animals must be genetically modified or treated with various interventions to perturb the metabolism to a state in which atherosclerosis occurs. Genetically modified mice with either ApoE^{-/-} [37] or LDLr^{-/-} [38] have perturbed lipid metabolism whereby elevated lipids leads to lipid deposition in the vessel wall and a form of atherosclerosis [21, 37, 39, 40]. As mentioned above, this process is accelerated by high fat and/or cholesterol feeding to generate atherosclerosis in a time frame consistent with animal experiments and practical laboratory requirements [41, 42]. It is also possible to supplement the treatments with STZ to induce hyperglycaemia but this generates mice of poor health and the utility of this, model may not be ideal in terms of mimicking the effect of diabetes on atherosclerosis in humans. The model may be improved by administering low-dose insulin to the mice [23].

Latest Developments in the Therapy of Diabetes and Associated Cardiovascular Disease

It is not widely appreciated that although there is a strong relationship between hyperglycaemia and the occurrence of cardiovascular disease events (e.g. heart attacks), most of the therapies for the treatment of hyperglycaemia and/or insulin resistance of diabetes have not resulted in a reduction in macrovascular disease (atherosclerosis) which manifests as a reduction in adverse cardiovascular events in clinical trials [43]. However, there has recently been what amounts to a very substantial change in this situation with the clinical trials of the SGLT 2 inhibitors. These drugs are derived from the family of phlorizin related sodium-glucose transport inhibitors. SGLT-2 inhibitors block the reabsorption of filtered glucose with a large loss of glucose in the urine and an accompanying hypoglycaemic actions. A recent study by Zinman and colleagues [9] showed that the SGLT-2 inhibitor, empagliflozin, reduced cardiovascular events and death relative to patients given placebo. It is interesting to observe that rather than event rates coalescing towards the end of the trial, the benefit of empagliflozin was increasing considerably and furthermore, the reduction in glycated haemoglobin levels was also maintained for 2 years (for data see Zinman et al., [9]). The substantial and highly statistically significant effect of empagliflozin at the end of the study period (42 – 48 months) was observed as a result of a rapid

increase in events in the control group which was prevented by empagliflozin. These drugs appear to be relatively very safe with the major side effect associated with the mechanistic appearance of glucose in the urine producing a favourable environment for the occurrence of urinary tract infections which is a modest unwanted effect compared, for example, to the severe, even lethal side-effects associated with the use of the PPAR γ ligands, the glitazones [44, 45]. There have also been very positive findings with drugs acting on the incretin system, glucagon like peptide (GLP 1) agonists [10, 11]. These agents have a prolonged and sustained anti-hyperglycaemic action and led to reductions in cardiovascular deaths and all cause deaths with good levels of safety (for details see semaglutide [10] and liraglutide [11]). This data for empagliflozin, semaglutide and liraglutide establishes a new baseline for the required efficacy of a new agent to treat the Type 2 diabetes and to reduce cardiovascular and all cause deaths.

So it is important to recognise that after decades of overlooking the area of diabetes, pharmaceutical companies and academic researchers invested an enormous effort in this area commencing 20 or so years ago and this has resulted in the identification of many potential new targets for diabetes. As mentioned above, several drugs have shown very beneficial effects in the treatment of diabetes and the reduction in deaths in large clinical trials. This changed environment needs to be taken into account when considering the discovery and validation of new therapeutic approaches to the treatment of diabetes.

One context addressed above is the treatment of the hyperglycaemia of diabetes to prevent its deleterious effects on the cardiovascular system and mortality, but another is the treatment of other cardiovascular risk factors, for example dyslipidaemia and hypertension, occurring in people with diabetes. In this context HMGCoA reductase inhibitors, or statins are amongst the most efficacious drugs in cardiovascular medicine – they block the production of cholesterol on liver cells leading to an upregulation of LDL uptake receptors and a clearance of lipid from the blood [46, 47]. Statins have been evaluated in many clinical trials – they are extremely efficacious but the limit of their efficacy is about 30 per cent [46, 47]. Statins are also very safe drugs with ironically a side-effect to raise blood glucose even to precipitate diabetes [48, 49]. Furthermore, the lipid lowering action may be accompanied by pleiotropic actions which complement the lipid-lowering action to reduce atherosclerosis [50, 51]. There are two very important corollaries – firstly, statins are the “gold standard” for the treatment of atherosclerosis and any new drug must add to the effect of a statin and cannot be claimed to replace statin usage – and secondly, with efficacy limited to 30 per cent there is a role for new therapies with a different mechanism of action which can be a combined product with a statin to provide an enhanced therapeutic efficacy with maintained safety [41, 42].

Conclusions

Diabetes is essentially a cardiovascular disease and drugs targeting the disturbed pathophysiology of diabetes must not only address these pathologies but also have a positive impact on the consequential effects on the cardiovascular system. There is no really good animal model available that allows for this to be undertaken in one experiment. This article suggests that it is necessary to look at to at least two models – one for the disturbed

metabolism in which as a minimum several doses of the test mixture are employed and both glucose and insulin levels are measured over time and one for the accelerated atherosclerosis in which the lipid deposition and plaque composition are assessed and in both cases suitable positive controls are utilised. Two specific models are suggested but there are a number of other models which might be equally useful. Thus, positive results from well-designed experiments in both these models would suggest that a drug intervention might have some possibility for having efficacy in the treatment of human diabetes and would underpin and validity of more research on any such product. In relation to the development of new medicines – traditional or Western type – it is necessary to be aware that the situation in relation to the efficacy of anti-hyperglycaemic agents has recently changed considerably. There are now at least two classes of drugs – GLP 1 agonists [10, 11] and SGLT 2 inhibitors [9] – that have been shown to in clinical trials to reduce hyperglycaemia and prevent death in people with diabetes and with a reasonable level of safety – this now represents the benchmark for the development of any new drug in this area. The main aim of this article has been to point out the complexities but also some of the solutions to the study and interpretation of investigations of TCM and natural products for potential therapeutic use in cardiometabolic diseases. A sophisticated approach to the evaluation of natural products for the treatment of cardiometabolic disease will expedite the discovery and development of new medicines in an area that has an exploding global population of people with diabetes.

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