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Review

Comparative aspects of rodent and nonrodent animal models for mechanistic and translational diabetes research

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A B S T R A C T

The prevalence of diabetes mellitus, which currently affects 387 million people worldwide, is permanently rising in both adults and adolescents. Despite numerous treatment options, diabetes mellitus is a progressive disease with severe comorbidities, such as nephropathy, neuropathy, and retinopathy, as well as cardiovascular disease. Therefore, animal models predictive of the efficacy and safety of novel compounds in humans are of great value to address the unmet need for improved therapeutics. Although rodent models provide important mechanistic insights, their predictive value for therapeutic outcomes in humans is limited. In recent years, the pig has gained importance for biomedical research because of its close similarity to human anatomy, physiology, size, and, in contrast to non-human primates, better ethical acceptance. In this review, anatomic, biochemical, physiological, and morphologic aspects relevant to diabetes research will be compared between different animal species, that is, mouse, rat, rabbit, pig, and non-human primates. The value of the pig as a model organism for diabetes research will be highlighted, and (dis)advantages of the currently available approaches for the generation of pig models exhibiting characteristics of metabolic syndrome or type 2 diabetes mellitus will be discussed.

1. Introduction

Diabetes mellitus is a major health burden, with 387 million people affected to date and an estimated increase to 592 million people by 2035 [1]. Currently, the prevalence of obesity as a major risk factor for diabetes mellitus is rising in adults, young children and adolescents. In 2013, almost one fourth of adolescents in developed countries were graded overweight or obese [2]. Furthermore, diabetes mellitus and diabetes prodromes, such as impaired glucose tolerance and insulin resistance, and abdominal obesity together with dyslipidemia and high blood pressure, are part of the so-called metabolic syndrome that encompasses the most dangerous risk factors for heart attack [1]. Despite numerous approved but often suboptimal treatment options, there is still no cure for any form of diabetes mellitus. Most diabetic patients, that is, 90% to 95%, suffer from type 2 diabetes, which is characterized by a combination of insulin resistance and insulin secretion defects, with variable manifestations resulting in relative insulin deficiency [3]. In addition, the ongoing epidemic of obesity has led to an increase in gestational diabetes mellitus (GDM). GDM, defined as carbohydrate intolerance of varied severity, develops around the 24th week of pregnancy in humans. It usually resolves after delivery but increases the risk for type 2 diabetes later in life [4]. In contrast, type 1 diabetes

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accounts for 5% to 10% of the diabetic population and is characterized by the immune-mediated destruction of insulin-producing beta-cells, leading to absolute insulin deficiency [3]. To address the unmet need for improved treatment options to combat or even cure diabetes mellitus, animal models closely resembling disease characteristics are urgently needed to predict the efficacy and safety of tested compounds in humans. Because of its multifactorial etiology and complex pathogenesis, no single animal model can model all characteristics of metabolic syndrome or type 2 diabetes mellitus. Rodent models have already provided important insights into disease mechanisms, but their predictive value for efficacy and safety of putative antidiabetic drugs in humans is limited [5]. In recent years, pigs have gained importance in biomedical research because of their close similarity to humans in many respects and because of the opportunities for genetic modification in this species. Most importantly, preclinical efficacy and safety studies in pig models are expected to be of particular translational value because of their human-like diabetic phenotype. Within the following sections, similarities and differences within different organ systems are compared between different animal species, and (dis) advantages of the currently available approaches for the generation of pig models of metabolic syndrome and type 2 diabetes mellitus are discussed.

2. Use of different animal species for diabetes research–differences and similarities

General characteristics of rodent, rabbit, pig, and non-human primate (NHP) models are summarized in Table 1.

To date, rodents represent the predominant species in biomedical research because of their low acquisition and maintenance costs (housing and food), standardized hygienic environment, good ethical acceptance, rapid reproductive biology (Table 1), efficient and well-established techniques for genetic modification, large-scale standardized phenotyping protocols, and the availability of a large database of reference information [18]. Although wild-type and genetically engineered rodent models have had a major impact on basic research, the translation of findings in rodent models to humans, for example, the prediction of clinical efficacy and safety of novel drug candidates, may be poor [5]. The second most common experimental animal species, the domestic rabbit (*Oryctolagus cuniculus*), also requires low maintenance costs and has good reproductive characteristics (Table 1). Despite several major differences compared to humans, for example, in terms of gastrointestinal tract physiology, the rabbit is a valuable model organism relevant for translational medicine (reviewed in [26]). Old World Monkeys, of which the most widely used species are cynomolgus monkeys (*Macaca fascicularis*) and rhesus monkeys (*Macaca mulatta*), are phylogenetically even closer to humans, but they have clear drawbacks as animal models. These include poor ethical acceptance, a long generation interval (Table 1), and the fact that they are mostly uniparous. Thus, the establishment of study groups with sufficient statistical power is costly and time consuming. Until recently, genetic tailoring of non-human primates (NHPs) was difficult, although the availability of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system for gene editing may change this situation [19]. Pigs have become attractive animal models as they share many anatomic, metabolic, physiological, and pathophysiological similarities with humans, have favorable reproduction characteristics (Table 1), and are ethically acceptable. An expanding repertoire of well-established techniques for the genetic modification of pigs, such as lentiviral transgenesis [27], nuclear transfer from gene-targeted cells [28], inducible transgene expression [29], and gene editing by site-directed nucleases [30-32], now facilitates the generation of tailored large animal models for diabetes research and other areas of translational medicine. Although genetic standardization can be achieved by inbreeding, for example, the Massachusetts General Hospital miniature pig [33], outbred pigs may more closely resemble the genetic variation of humans. In the following chapters, we discuss aspects related to animal size and species-specific differences and similarities in diabetes-related organ systems and consequences for studies related to diabetes prevention, for example, nutritional intervention or drug efficacy and safety.

2.1. Size matters

The small size of rodents and rabbits is advantageous for maintenance costs and the small amount of test compound needed, but it limits the sample material available per animal, particularly in rodents. Minipigs, such as the Göttingen minipig, represent a compromise between rodents and domestic pigs and are therefore widely used for efficacy and safety studies of drug candidates [34]. Generally, only pigs match the size and weight of humans over a wide range of developmental periods. Domestic pigs cover infancy and adolescence and are suitable for short- and medium-term studies (up to several months) because of their rapid growth, whereas minipig breeds cover adulthood and are ideal for long-term studies (several months up to years) once they are in the non-growing adult phase. Pig size often allows for the direct transfer of medical devices, for example, bioartificial pancreas [35], surgical techniques, for example, bariatric surgery [36], and percutaneous catheter interventions for revascularization (reviewed in [37]) or non-invasive imaging techniques (ultrasonography, computed tomography, and magnetic resonance imaging) from experimental studies to clinical application in human patients. In contrast to pigs, non-invasive imaging techniques in rodents still have resolution limitations because of their small size and even ultrasonography requires anesthesia [38]. Moreover, non-invasive imaging approaches for the quantification of islet/beta-cell mass (reviewed in [39]) can be reliably tested in human-sized pig models, for example, reduced beta-cell mass [40,41] or diet-induced obesity [42], and subsequently be validated by quantitative stereological analyses of the pancreas [41]. The large blood volume of pigs (Table 1) enables the reliable performance of metabolic tests, for example, glucose/insulin tolerance tests and clamp studies, with frequent recovery of blood samples large enough to perform complex hormone and metabolite profiling in each sample. Moreover, blood samples can be taken without difficulty from pig fetuses or