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Opinion

Bias in Application of Animal Models for Diabetic Research

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Therapeutic approaches to treat diabetes mellitus (DM) are screened in animal models, as described in recent [1]. Generally, the mammalian models are applied to study food intake, body weight and/or fat distribution, glucose metabolism, or other aspects of metabolic homeostasis.

The monogenic animal models of metabolic diseases are widely applied in the new drug development. A lack of leptin production (*ob/ob*) or lack of leptin sensing (*db/db*) in mouse belonged to mostly used model. The hyperglycemic obese Zucker (ZDF) rats and Otsuka Long-Evans Tokushima Fatty (OLETF) rats are also popularly used in diabetic research. Interestingly, more than 50 genetic loci associated with the phenotypic parameters of glucose homeostasis, metabolism or obesity were observed from a systemic phenotyping screen [2]. Therefore, new monogenetic animal models showing diabetes or obesity will be available in the near future.

In clinics, type 1 DM (T1DM) and type 2 DM (T2DM) are mostly diagnosed, although they have different causes, both due to pancreatic β-cell dysfunction. Therefore, damaging the pancreas chemically or mechanically is widely applied to induce the diabetic models. Surgical removing of pancreatic tissue (pancreatectomy) has initially been used to induce diabetic disorders [1]. The lack of toxic effects on another organ belonged to the advantage of this method. However, several factors, including the special training and surgical equipment in addition to the adverse effects on pancreatic enzymes or hormones, limit the application of pancreatectomy in basic research. Therefore, the chemical toxins, such as streptozotocin (STZ) or alloxan, targeting pancreatic tissue are developed. In diabetic research, STZ is applied more widely than alloxan due to less toxic and more stable. Both toxins have been used to induce the model of T1DM through the critical damage of pancreatic β-cells. Additionally, both toxins at low dose elicit a partial damage of islet only, similar to the models of T2DM.

Recently, a popular model is to combine high-fat diet (HFD) feeding with a subsequent intraperitoneally injection of STZ at a low dose (30 - 40 mg/kg) to induce the model of T2DM [3]. The advantage of this model resembles the progress of T2DM that occurs in clinics, due to the slow effect of HFD to induce glucose intolerance and insulin resistance, followed with an increase in plasma glucose using STZ-induced pancreatic injury. The symptoms of hypoinsulinemia and hyperglycemia are widely achieved by STZ through single or multiple injections at low-dose. Protocols are being continuously refined and likely differ between species and even strains, as mentioned in a review article [1]. Also, the adverse effects of STZ including liver and/or renal toxicity and carcinogen-like activity shall be concerned.

Insulin resistance (IR) is one of the major characteristics of T2DM. the animal models of IR have been introduced in a review article [8]. Similar to diet-induced DM, rats fed a high fructose diet showed a tendency of slight IR at week 4 and a marked hyperinsulinemia or IR after 8 weeks [4]. However, the diet manipulated models need a long time to induce the DM. Therefore, a more rapid model using the administration of STZ with nicotinamide was developed [5] because this STZ + nicotinamide model can be easily induced within just a few days. Depending on the presence of hyperglycemia and/or hypoinsulinemia, this model has also been widely used in T2DM research for a while. Moreover, the diabetic model induced by the injection of STZ at a low dose is another model of T2DM showing β-cell failure without obesity [6]. We have compared these 4 models and suggest that fructose-fed rats belong to pre-diabetic in clinics. Additionally, depending on the hyperglycemia and degree of IR, rats received nicotinamide + STZ injection belong to the initial stage of diabetes followed by the rats injected STZ at low dose. The rats induced by HFD+STZ seem close to the clinical cases at the stage later than the low dose STZ-treated rats [7]. However, a longer time for induction and the continuous supply of HFD during the study belong to the weakness of HFD+STZ model.

Mainly, selecting an appropriate animal model for diabetic research is essential. For drug development in preclinical studies, as described previously [1], the susceptible polygenic models seem helpful to screen the compound efficacy and safety. For the subsequent validation of novel therapeutics, the genetic loss-of-function models are the more relevant, although the economic problem(s) shall be concerned together.

During the application of animal models, bias is easily to elicit by the researchers. Basically, as described previously [9], one perhaps underestimated factor that may contribute to the generation of unreliable results is unconscious bias. The bias is based on experiences, prejudices, and many other factors. It is necessary to be aware of bias in order to limit or possibly prevent it. Examples regarding the bias in the application of animal models are introduced below.

First, no animal model is fully the same as human disorders. Each model was developed to reach and/or mimic the human disease in a specific target. For example, the popularly used T2DM model of HFD+STZ in rats or mice showed IR or metabolic disorders but it is still far from the clinical disorders of T2DM. Therefore, results from the animal model may contribute the evidence to know the specific disorder(s) associated with the model. It is not suitable to conclude that merits in HFD+STZ rats or mice are the same as that in human T2DM.

The monogenic models of diabetic animals show metabolic disorders after the matured development mostly. Therefore, the application of genetic mice needs to take care of the age and identify the success of the model. For example, Zucker rats suffer diabetic disorders after 12 weeks old. They were generally used in two divisions between 8-week-old group (normal animals) and 13-week-old group (diabetic animals). For screening the new agent or drug, only the Zucker rats aged 12-week over could be applied. Additionally, mice due to a lack of leptin production (ob/ob) or lack of leptin sensing (db/db) shall be used carefully in the same manner.

The dose of toxin used to induce diabetic model needs to follow in a correct way. Such as, STZ + nicotinamide model must treat nicotinamide at the dose of 230 mg/kg through intraperitoneal injection 15 minutes before an intravenous injection of STZ (65 mg/kg), as described previously [5]. But, some reports applied the different dose of nicotinamide: one report used nicotinamide at 60 mg/kg [10] and another one used 110 mg/kg [11] to induce the model. Then, a bias model showing hyperglycemia, fasting blood glucose over 410 mg/dl [10] and 420 mg/dl [11] in a way markedly varied with the original report [5], was prepared. Both were similar to the T1DM-like model, probably due to the failure of nicotinamide to compensate for the damage of STZ in the pancreatic islet. Moreover, the model of HFD+STZ in rats or mice was also modified by researchers. Originally, it is indicated to induce via 40% HFD for two weeks and intravenous injection of STZ at 50 mg/kg [3]. But, it was modified to 58% HFD for two weeks and intraperitoneal injection of STZ (35 mg/kg) and published at 5 years later [12]. Additionally, may study using another modified way were also published while the main factors including the composition of HFD (percentage) and the treatment of STZ (dose and treated method) were not conducted in clear. Therefore, bias is included in many reports that could be found easily. Otherwise, fasting time and animals under anesthesia or not may also influence the induction of model. Overall, alternation is easily elicited by the researchers majored in another field due to unconscious and/or less experience in the model induction. Then, the prepared article is reviewed by the referee majored in the same field during submission. Modification in model induction has not been evaluated by the reviewer to result in the bias shown in the published paper.

Therefore, bias in the application of animal models for diabetic research is easy to elicit in an unconscious manner. It is necessary to be aware of bias in order to prevent it. The major way is carefully checking by the researcher(s) during design the project or proposal. Also, the reviewer may assist to limit it during the submission using individual experience.

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