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Experimental diabetes induced by alloxan and streptozotocin: The current state of the art

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder with a high prevalence worldwide. Animal models of diabetes represent an important tool in diabetes investigation that helps us to avoid unnecessary and ethically challenging studies in human subjects, as well as to obtain a comprehensive scientific viewpoint of this disease. Although there are several methods through which diabetes can be induced, chemical methods of alloxan- and streptozotocin-induced diabetes represent the most important and highly preferable experimental models for this pathological condition. Therefore, the aim of this article was to review the current knowledge related to quoted models of diabetes, including to this point available information about mechanism of action, particular time- and dose-dependent protocols, frequent problems, as well as major limitations linked to laboratory application of alloxan and sterptozotocin in inducing diabetes. Given that diabetes is known to be closely associated with serious health consequences it is of fundamental importance that current animal models for induction of diabetes should be continuously upgraded in order to improve overall prevention, diagnosis and treatment of this pathological condition.

1. Introduction

Diabetes is a chronic metabolic disorder, which appears when there is a defect in insulin secretion or/and when the body cannot use insulin in an effective way. This leads to an increase in blood level of glucose, which is called hyperglycemia. Two major forms of diabetes are generally identified, namely diabetes type 1, also known as juvenile-onset diabetes, and diabetes type 2, formerly termed as adult-onset diabetes (Cefalu, 2006). Type 1 diabetes represents approximately 10% of all cases of diabetes, and it develops as a result of autoimmune process in which B cells destruction is a final consequence of a T-cell-mediated autoimmune attack (Mathis, Vence, & Benoist, 2001; Rother, 2007). This type of diabetes results from the pancreas failure to produce insulin and the person with this type of diabetes requires regular insulin injections, which is why this type is also known as an insulin-dependent diabetes mellitus. The main characteristics of type 1 diabetes are high glucose and low insulin levels in blood. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, and it represents more than 90% of all cases (Cefalu, 2006). This type is known as a progressive disorder, and it is associated with gradual diminishing of pancreatic function over a period of time. Although type 2 diabetes can be sometimes concomitantly found with an absolute insulin deficiency, at the beginning, the main problem is usually not insulin secretion but the peripheral resistance to insulin. This type can also progress toward insulin dependency during a certain period of time. This is why type 2 diabetes can be associated with elevated, normal or low insulin levels (Cefalu, 2006). Apart from these two types of diabetes there is an additional type called gestational diabetes which occurs in pregnant women, who never experienced any problem with high blood glucose level before pregnancy (Radenković, 2011). Additionally, gestational diabetes is considered to be very important since it can frequently precede development of type 2 diabetes (Kumar, Singh, Vasudeva, & Sharma, 2012; Radenković, Grbovic, Radunovic, & Momcilov, 2007).

Over the recent years the prevalence of diabetes throughout the world population has increased dramatically, unfortunately this trend will continue in the future. This rise in prevalence of diabetes can be explained with several factors, including overall population growth, aging, urbanization and an increase of obesity and physical inactivity (Kumar et al., 2012). In order to better understand the significance of this increase in prevalence of diabetes, it is important to note that the number of people affected by diabetes in 1980 was 153 million and that this number significantly increased to 347 million in the year 2008 (Danaei et al., 2011). This is indeed a major public issue because diabetes is a

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complex, multifactorial disease and the overall risk of dying among people with diabetes is at least double, if comparing to the risk of their peers without diabetes (Roglic et al., 2005). The economic costs of medical care provided for patients with diabetes, as well as related complications are stupefying. For example, in 2002 it was estimated that direct and indirect medical expenditures related to diabetes were 132 billion US dollars, whereas in 2007 this number was even higher and the estimated costs were 174 billion US dollars (Dall, Nikolov, & Hogan, 2003; American Diabetes Association, 2008). Finally, in 2012 the estimated total economic cost of diagnosed diabetes was 245 billion US dollars, which is a 41% increase from the previous estimation reported in 2007 (American Diabetes Association, 2013). Concerning these assessments, it is easy to comprehend the substantial burden that diabetes imposes on society.

Considering previously mentioned facts, it is clear that well designed researches in the field of diabetes are needed in the future to obtain new knowledge that will be used to reduce the consequences associated with this disease. Although the epidemiology of diabetes has been well described, there is much more about the pathological process itself that we need to learn. For example, we need to find out more about complex mechanisms that underlie the development of diabetes and its complications, to understand the natural history of the disease, to identify new targets for therapy, and to re-evaluate already existing interventions and treatments. Although some of the previously mentioned problems can be resolved by further studies in human population, unfortunately there are many questions that can be answered only through invasive manipulations or observations that are not allowed in humans either for logistical or ethical reasons (Kaplan & Wagner, 2006). A major concern in investigation of diabetes is its natural history that takes years to be developed, as well as time needed before all complications would be detectable. Also, it is time consuming and very expensive to assess the effect of different interventions aimed to modulate development of diabetes or its complications (Cefalu, 2006). To address this concern, it is also important to summarize current knowledge about existing animal models of diabetes, and if possible to develop and utilize new ones in order for planed interventions to be assessed in much shorter time spans. Accordingly, the aim of this review paper was to collect and present all the important facts concerning chemically induced diabetes with streptozotocin and alloxan, and to point out all positive and negative characteristics of these experimental diabetes models.

2. Ethical issues of animal use in diabetes mellitus research

Whenever animal research is mentioned, including diabetes research in animals, two important opinions can be distinguished (Rees & Alcolado, 2005). One of them is that animals are unable to provide informed consent and have no direct or indirect benefit from the experiments, while the other opinion points out those experiments on animals should be performed with little or no external influence. The truth is that animal research is essential for obtaining many conclusions that can help us in everyday medical practice and also to reduce human and financial impairments. On the other hand, animal use in research should not be taken for granted, since this could be a serious ethical issue (Radenković, 2012). The ethical problem is regarded in the fact that the use of animals in experiments, can also involve the suffering of the animals, which can be physical and/or psychological (Levy, 2012). This means that existing similarities between humans and animals cannot entirely justify the use of animals for research, since those similarities also raise some ethical issues, given that as humans, animals are also able to suffer. Numerous efforts have been made to replace needless use of animals with alternative in vitro models. This is with the intention of to reducing the number of animals used in experiments and to reduce the pain and discomfort (Kroeger, 2006). One of the most important regulation concepts of animal welfare is the *3Rs* concept (replacement, reduction and refinement) created by Russell and Burch in 1959 (Kroeger, 2006). The *3Rs* concept allows fair use of animals in research and the correct application of this model reduces the possibility of compromising overall animal welfare. Also, a good scientist should never forget that the "best animal welfare" results in the "best science" (Russell & Burch, 1959).

When ethics of utilizing animal models of diabetes are concerned, it is important to realize that advances in biomedical sciences usually come as a combination of results obtained from all levels of research, from molecular to clinical, and that animal use is only one of the steps in obtaining these results. Also, one should always bear in mind that animal models of diabetes in particular have provided many benefits to humans including discovery of insulin. Nevertheless, this does not mean that animal use in diabetes-related investigations should not be justified at all times. Moreover, before the start of any research one must establish if that investigation will provide substantial benefits, such as saving of human lives, or the considerable contribution to human welfare (Hoff, 1980). The major problem is that biomedical studies in which animals are used do not always provide an immediate benefit to humans (Sieber & Traystman, 1993), and this is something that makes the real challenge, not only in diabetes research, but in all other fields that include use of laboratory animals.

3. Animal models of diabetes

Animal models have been used extensively to obtain different information about various pathological conditions. Until now, many animal models of diabetes have been created. For an animal model of diabetes it is important that it can mirror the pathogenesis and natural history of diabetes or/and to induce further development of specific complications connected to it. However, the fact is that we cannot underline any single animal model of diabetes to be entirely suitable in terms of mimicking development of human diabetes and its complications. Since there is no animal model of diabetes that encompasses all of the above mentioned, it is desirable to continue with the development of new improved models of diabetes that will completely, or at least more adequately than current models, mimic different aspects of human diabetes.

In earlier animal studies, a model of pancreatectomized dog was used in order to confirm the central role of the pancreas in glucose homeostasis, as well as for discovery and purification of insulin (Lenzen & Panten, 1988; Rees & Alcolado, 2005). In fact, pancreatectomy was the first animal model of diabetes. Since that period many new animal models of diabetes have been created. Nowadays, experiments are rarely performed on dogs for many reasons. One of them is based on the guiding principles for animal research directing us to utilize the lowest level of animal species available. Also, the smaller the animal is, the more convenient and less expensive the experiment will be. This is why the majority of experiments are performed on rodents. It is important to note that rats are the first choice of use, comprising over 85% of these models (Wilson & Islam, 2012). Nevertheless, one should bear in mind that for example according to the Annual Statistics of Scientific Procedures on Living Animals Great Britain 2013 (2014) rodents are the most commonly used animals for scientific research, and that at the same time mice are generally far more frequently used than rats. This can also be the case in diabetes investigations since mice are more appropriate for developing of transgenic strains. Although rodents are the most commonly used species, they are not completely ideal for diabetes research. One of important issue is that they may not adequately reflect various human homeostatic mechanisms (Rees & Alcolado, 2005). For this reason some larger animals including cats, dogs, pigs and primates are also used. Accordingly, domestic cats have been used because this is one of the few outbreed models characterized by insulin resistance, defective insulin secretion, islet amyloid formation, and B cells loss (Henson & O'Brien, 2006). Moreover, amyloid formation in the pancreas is one of the main pathological findings in primates (Wagner et al., 2006), which is important