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Review Article

DIFFERENT ANIMAL MODELS FOR DRUGS WITH POTENTIAL ANTIDIABETIC PROPERTIES

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ABSTRACT
The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, largest number of people with diabetes. Although medicinal plants have been historically used for diabetes treatment throughout the world, few of them have been validated by scientific criteria. In recent times, an outsized multiplicity of animal models has been developed to enhanced understand the pathogenesis of diabetes mellitus and new drugs have been introduced in the market to treat this disease. The aim of this work was to review the available animal models of diabetes and some in vitro models which have been used as tools to investigate the mechanism of action of drugs with potential Antidiabetic properties.
KEYWORDS: Murine, Alloxan, AE, AT, i.p, p.o, STZ

INTRODUCTION

In the development of new drugs to prevent the burden of complications associated with diabetics and the raised interest in the scientific community to evaluate either raw or isolated natural products in experimental studies, few of them were tested in humans. Nevertheless, natural supplements are widely used around the world to treat diabetes, but medical research does not support their effectiveness. In these studies the low mechanical quality, small sample size of tested patients, and limited number of trials deserve concern in the explanation of the positive data and require auxiliary examination in high-quality trials. Nowadays, clinical treatment of diabetes targets both insulin deficiency and resistance and more recently the prevention of pancreatic cell function decline. By definition, diabetes mellitus is categorized as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The vast majority of cases of diabetes fall into two broad pathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response. The intention of this work was to review the available animal models of diabetes in order to make available sufficient gear to investigate the mechanism of action of drugs with potential antidiabetic properties. The decision of which model of diabetes to use for any particular protocol is mainly influenced by local resources. Ideally, preclinical experiments should be initially carried out in vivo, and be complemented, when possible, with in vitro studies to explore and advance in the mechanism of action of a natural product. Diabetes can be induced by pharmacologic, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals. The classical model employed by Banting and Best was pancreatectomy in dogs. It is also described prone strains to diabetes mellitus that have been employed in several researches. Currently, the murine model is one of the most used due to the availability of over 200 well-characterized inbred strains and the ability to delete or over-express specific genes through knockout and transgenic technology [1-3] (Fig.1-2)

IN VIVO ANIMAL MODELS OF DIABETES MELLITUS (Fig.3)

Pharmacological induction of diabetes

The majority of studies published models includes Streptozotocin (STZ, 69%) and alloxan (31%) induced animal models are by far the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status. According to the

administered dose of these agents, syndromes similar to either type 1, type 2 diabetes mellitus or glucose intolerance can be induced. Protocols are available anywhere, being critical the pH and type of buffer employed as well as the preparation of the solution of either alloxan or streptozotocin in the day of the experiments. By using these models of diabetes induced by chemical drugs, the majority of published studies report the amount of reduction of blood glucose that is always evaluated after a period of fasting following acute or chronic treatment with a specific natural product. Comparative studies are carried out with nondiabetic and/or diabetic animal groups treated with known antidiabetic drugs, but results do not permit to further explore the mechanism of action of the studied natural product. Table 1 displays a list of few plants and/or their active compounds tested in diabetic animals (induced by either STZ or alloxan) published in the past years.[4-5] (Table.1)

Surgical models of diabetes

The action or relative glucose uptake in various tissues of 90% pancreatectomized rats by using either hyperglycemic or euglycemic hyperinsulinemic clamp method. This experimental design permits to evaluate if the compound has some effect upon both resistance to and secretion of insulin. Another technique used to induce diabetes is the complete removal of the pancreas. Few researchers have employed this model in the last years to explore effects of natural products with animal species such as rats, pigs, dogs and primates. Limitations to this technique include Major surgery and high risk of animal infection, High level of technical expertise and adequate surgical room environment, Supplementation with pancreatic enzymes to prevent malabsorption, Adequate post-operative analgesia and antibiotic administration, Loss of pancreatic counter regulatory response to hypoglycemia. More recently, partial pancreatectomy has been employed, but large resection (more than 80% in rats) is required to obtain mild to moderate hyperglycemia. In this case, small additional resection can result in significant hypoinsulinemia.[6-8]

Genetic models of diabetes

Genetically engineered diabetic mice

Although significant advances in this field have arisen in recent years, especially with the advent of transgenic mice, there have been no studies carried out involving natural products and these models. Certainly, the high costs restrict their study in sophisticated protocols which explore mechanisms of potential therapeutic agents that either stimulate pancreatic β -cell growth or inhibit pancreatic β -cell death. In this case, rodents may be produced to over (transgenic) or under (knockout)-express proteins thought to play a key part in glucose metabolism.

Animal strains that spontaneously develop diabetes

These models allow the estimate of the effect of a natural product in an animal without the hindrance of side effects induced by chemical drugs like alloxan and STZ reported above. Similar to the human condition, these strains display complex and heterogeneous characteristics. In some of these models, insulin resistance predominates in association with obesity, dyslipidemia and hypertension, which provides valuable insights to study some events that are observed in human type 2 diabetes mellitus. Conversely, some strains like Ob/Ob mouse may maintain euglycemia due to a robust and persistent compensatory pancreatic β -cell response, matching the insulin resistance with hyperinsulinemia. On the other hand, the db/db mouse rapidly develops hyperglycemia since their pancreatic β -cells are unable to maintain the high levels of insulin secretion required throughout life.[9-10]

Other models of type 2 diabetes

A method to induce diabetes in adult rats is to mimic the unfavorable intrauterine environment, which in humans leads to low-birth weight and is supposed to confer high risk for the development of diabetes in adult age. This model known as intrauterine growth retardation by uteroplacental insufficiency in the rat is based on the premise that uterine malnutrition may also increase the risk of diabetes amongst offspring in later life . This has been achieved by several means, including bilateral uterine artery ligation at 19 days of gestation, i.e. 3 days before term. The diabetogenic effects of manipulating the intrauterine environment are probably mediated by a permanent programming of the developing offspring, e.g. by the mechanism of imprinting. It should also be pointed out that the increased risk of diabetes continues into subsequent generations, which in turn, suggests that changes also affect the germ cell line.[11]

IN VITRO STUDIES:

In vitro studies on insulin secretion

Incretins, and transcription factors such as peroxisome proliferator-activated receptors—PPAR are targets of modern therapy. Insulin receptor, glucose transporters, however, has not been yet the focus of antidiabetic therapy.

Studies using isolated pancreatic islet cell lines

In type 2 diabetes, pancreatic β -cells exhibit atypical ion channel activity and an abnormal pattern of insulin secretion . These pathways can be studied with isolated pancreatic β -cells from either control or diabetic rat or mouse that can be obtained by collagenase digestion technique, followed by adequate separation and

transference to appropriated culture medium.

Several in vitro assays are available to study different steps of insulin secretion. It is known that insulin secretion occurs when pancreatic β -cells utilize glucose to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage-dependent Ca^{2+} channels. This results in elevation of the intracellular Ca^{2+} concentration which triggers insulin secretion afterwards, the experimental protocol is assayed.[12]

Studies using insulin-secreting cell lines

Bioengineered technologies have provided new opportunities to improve and establish more appropriate cultured cell lines to help to facilitate studies of mechanisms of both insulin secretion and β -cell dysfunction, being also the target to the study of natural products. The most widely used insulin-secreting cell lines are RIN, HIT, beta-TC, MIN6 and INS-1 cell. These cell lines release mainly insulin and small amounts of glucagon and somatostatin. Although the behaviour of none of these cell lines perfectly mimics primary β -cell physiology, they are extremely valuable tools for the study of molecular events underlying β -cell function.[13]

In vitro studies on glucose uptake

Adipose tissue is considered a key link between obesity and type 2 diabetes by promoting the development of lipotoxicity, i.e. cell damage as a consequence of elevated intracellular lipid concentrations and insulin resistance . Insulin resistance either at the adipocyte or skeletal muscle levels contribute to hyperglycemia. However, adipocytes from different sites of the body may have different biological or pathological effects. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as murine 3T3-L1 cells and rat L6 muscle engineered to over-express GLUT4 and may be employed as tools to evaluate the effects of natural products upon glucose uptake.[14-15]

CONCLUSION

At this time, a standard model of experimental diabetes to study effects of drugs, which could help in preventing the progressive loss of pancreatic islet function remains to be established. In spite of the worldwide use of herbs and medicinal plants, the effective treatment of diabetes with phytochemicals has not been validated with scientific criteria which may support their substitution for the current therapy. Thus, by spotlighting new models may help to elucidate effects of medicinal plants employed in the treatment of diabetes mellitus. This review contributes to the researcher in the pharmacology field to designs new strategies for the development of fresh drugs to treat this serious condition that constitutes a global public health.

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Table 1 List of few plants and their active compounds test

Plant (family)	Material	Treatment	Drug-induced diabetes	Results	References
Aegle marmelos (Rutaceae)	EtOH/leaves AE/seeds	i.p., 14 d p.o., 14 d	STZ-rat STZ-rat	↓Glucose, ↓glycosylated hemoglobin, ↑C-peptide, ↑glucose ↓Glucose	Narendhirakannan et al. (2006) Kesari et al. (2006)
Aloe vera (Liliaceae)	EtOH/leaves	p.o., 21 d	STZ-rat	↓Glucose, ↓lipids	Rajasekaran et al. (2004)
Bryophyllum pinnatum (Crassulaceae)	AE/leaves	p.o./i.p., AT	STZ-rat	↓Glucose	Ojewole (2005)
Chamaemelum nobile (Asteraceae)	AE/leaves	p.o., 15 d	STZ-rat	↓Glucose	Eddouks et al. (2005)
Eugenia jambolana (Asteraceae)	AE, EtOH, isolated compounds/fruitpulp/ seeds	p.o., AT	STZ-rabbit	↓Glucose, ↓lipid, ↑glucose tolerance	Sharma et al. (2006); Ravi et al. (2005)
Garlic (Allium sativum L.) (Liliaceae)	EtOH/bulbs	p.o., 14 d	STZ-rat	↓Glucose, ↓lipids, ↑insulin	Eidi et al. (2006)
Momordica charantia (Cucurbitaceae)	Fruits	p.o.,21 d	Alloxan-rat	↓Glucose, ↓lipids	Yadav et al. (2005)
Salvia officinalis (Lamiaceae)	AE/leaves	p.o., 14 d	STZ-rat, mice	↓Glucose ↓gluconeogenesis	Lima et al. (2006)
Terminalia chebula (Combretaceae)	CH_3Cl extract/seeds	p.o., AT	STZ-rat	↓Glucose	Rao and Nammi (2006)

AE: aqueous extract, AT: acute treatment, CH_3Cl: chloroform extract, EtOH: ethanolic extract, i.p.: intraperitoneal route, MeOH: methanolic extract, p.o.: oral route, and STZ: streptozotocin.

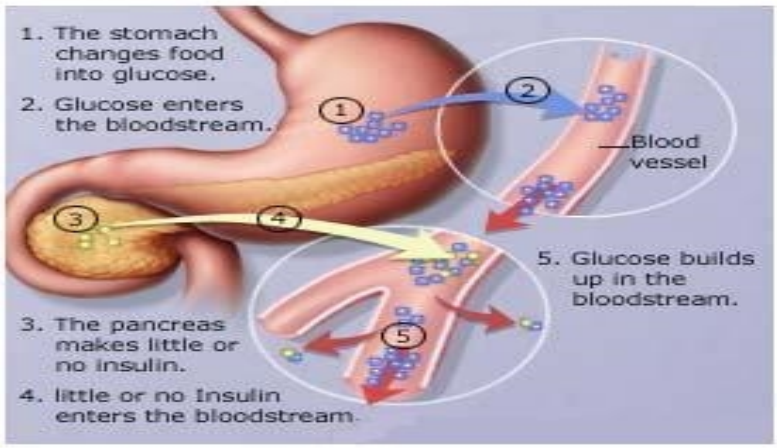

Figure.1 Diabetes

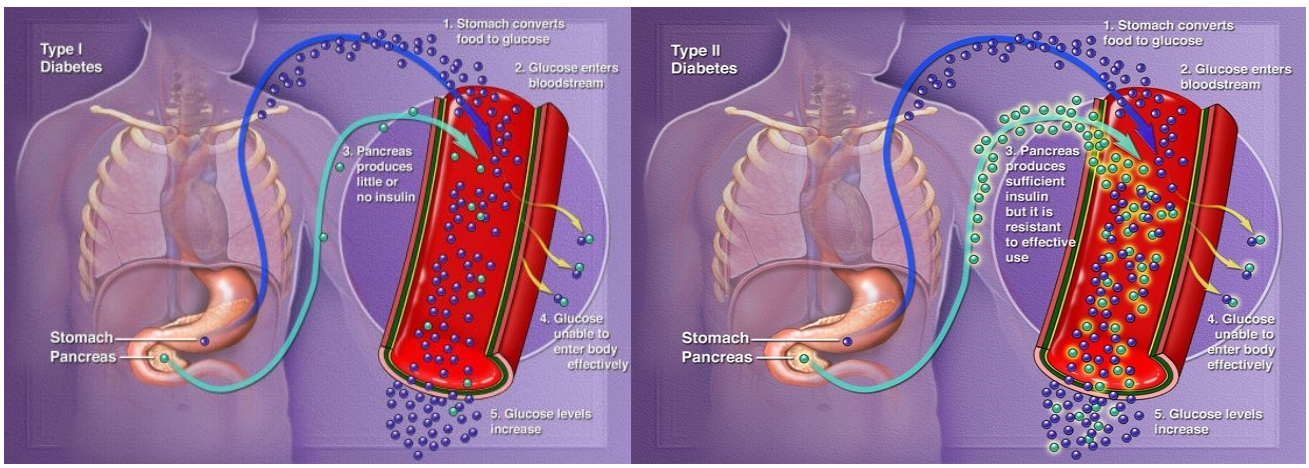

Figure.2 Type1 and Type2 Diabetes

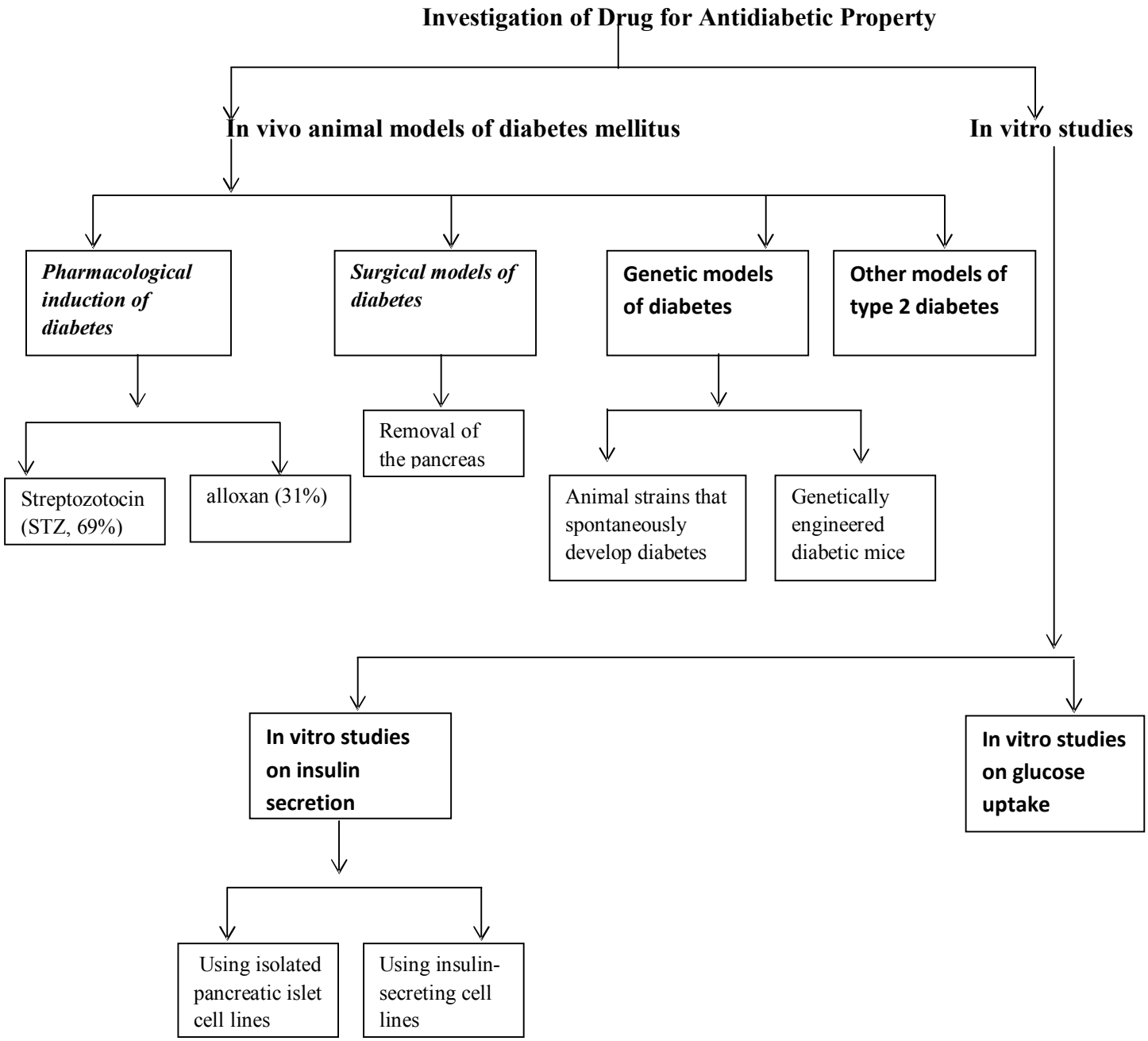

Figure.3 Animal Model