Animal models to test drugs with potential antidiabetic activity

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Abstract

Although medicinal plants have been historically used for diabetes treatment throughout the world, few of them have been validated by scientific criteria. Recently, a large diversity of animal models has been developed to better 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. In addition, a MEDLINE/PUBMED search for articles on natural products, pancreatectomy and diabetes mellitus treatment published between 1996 and 2006 was done. In the majority of the studies, natural products mainly derived from plants have been tested in diabetes models induced by chemical agents. This review contributes to the researcher in the ethnopharmacology field to designs new strategies for the development of novel drugs to treat this serious condition that constitutes a global public health.

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Keywords: Diabetes mellitus; Animal models; Genetic studies; Natural products

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Abbreviations: ADP, adenosine diphosphate; AE, aqueous extract; AT, acute treatment; ATP, adenosine triphosphate; BB rat, Bio Breeding Laboratories rat; BuOH, n-butanol fraction; Ca2+, calcium; CH2Cl2, methylene chloride extract; CH3Cl, chloroform extract; db/db mouse, monogenic model of obesity (leptin resistant); DNA, deoxyribonucleic acid; DPP-4, dipeptidyl peptidase-4; EtOAc, ethyl acetate fraction; EtOH, ethanolic extract; GK rat, Goto-Kazikasi rat; GLUT, glucose transporter; Hex, hexane fraction; i.p., intraperitoneal route; i.v., intravenous route; IGFs, insulin-like growth factors; MeOH, methanolic extract; NOD mouse, non-obese diabetic mouse; Ob/Ob mouse, monogenic model of obesity mouse (leptin deficient); OLETL rat, Otsuka Long-Evans Tokushima Fatty rat; p.o., oral route; STZ, streptozotocin; ZK rat, Zucker rat.

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1. Introduction

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes (Wild et al., 2004). 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 etiopathogenetic 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 (American Diabetes Association, 2005).

Despite the great interest in the development of new drugs to prevent the burden of complications associated with this disease and the raised interest in the scientific community to evaluate either raw or isolated natural products in experimental studies (Fig. 1), few of them were tested in humans (Liu et al., 2004; Vuksan and Sievenpiper, 2005; Johnson et al., 2006; Jung et al., 2006). Nevertheless, natural supplements are widely used around the world to treat diabetes, but medical research does not support their effectiveness. In these studies the methodological quality, small sample size of tested patients, and limited number of trials deserve caution in the interpretation of the positive data and require further examination in high-quality trials (Liu et al., 2004). Nowadays, clinical treatment of diabetes targets both insulin deficiency and resistance and more recently the prevention of pancreatic β-cell function decline (Hansotia and Drucker, 2005) (Table 1).

The aim of this work was to review the available animal models of diabetes in order to provide adequate tools to investigate the mechanism of action of drugs with potential antidiabetic properties. Considering that there is a marked overlap between type 1 and 2 diabetes mellitus either in human beings or in animal models, some classical models for each type of this disease cannot also be sustained (Chaparro et al., 2006; Donath and Ehses, 2006). However, it is cited, when appropriated along the text, which category (type) the described model fits better.

The information in this review was obtained by searching MEDLINE/PUBMED for literature published in English from 1996 to 2006, using terms natural products, pancreatectomy and diabetes mellitus treatment. 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.

2. In vivo animal models of diabetes mellitus

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 (Bliss, 2000). It is also described prone strains to diabetes mellitus that have been employed in several researches (Chen and Wang, 2005; Rees and Alcolado, 2005; Masiello, 2006). 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 technologies (Rees and Alcolado, 2005; Masiello, 2006).

2.1. Pharmacological induction of diabetes

The majority of studies published in the field of ethnopharmacology between 1996 and 2006 employed these models. Streptozotocin (STZ, 69%) and alloxan (31%) 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 (Lenzen et al., 1996; Mythili et al., 2004). Protocols are available any where, 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 (Yu et al., 2000; Gupta et al., 2005a,b; Lei et al., 2005; Babu et al., 2006; Miranda et al., 2006).
The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species, but both drugs differ in their mechanism of action (Federiuk et al., 2004; Lei et al., 2005).

Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreatic β-cells (Szudelski, 2001). The range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic and may cause the loss of many animals. This loss is likely to stem from kidney tubular cell necrotic toxicity, in particular when too high doses of alloxan are administered (Lenzen et al., 1996). The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally (i.p.) or subcutaneously its effective dose must be higher (Federiuk et al., 2004). For instance, an intraperitoneal dose below 150 mg/kg may be insufficient for inducing diabetes in this animal species (Katsumata et al., 1992). In mice, doses vary among 100–200 mg/kg by intravenous route (i.v.) (Machado et al., 2001; Miranda et al., 2006).

Streptozotocin enters the pancreatic β-cell via a glucose transporter-GLUT2 and causes akylation of deoxyribo nucleic acid (DNA). Furthermore, STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic β-cells are destroyed by necrosis (Mythili et al., 2004). In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes (Patel et al., 2006), but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single doses below 40 mg/kg may be ineffective (Katsumata et al., 1992). In general, rats are considered diabetic if tail blood glucose concentrations in fed animals are greater than 200–300 mg/dl, 2 days after STZ injection. A model of type 2 diabetes can be induced in rats by either i.v. (tail vein) or i.p. treatment with STZ in the first days of life. At 8–10 weeks of age and thereafter, rats neonatally treated with STZ manifest mild basal hyperglycemia, an impaired response to the glucose tolerance test, and a loss of pancreatic β-cell sensitivity to glucose (Pascoe and Storlien, 1990). It has been observed that STZ at first abolished the pancreatic β-cell response to glucose, but a temporary return of responsiveness then appears which is followed by its permanent loss (Mythili et al., 2004). In adult mice, STZ given in multiple low doses (40 mg/kg, i.v. for 5 days) (Rees and Alcolado, 2005) induces an insulin dependent diabetes that is quite similar to the autoimmune forms (islet inflammation and β-cell death) of type 1 diabetes. On the other hand, a single dose between 60 and 100 mg/kg of STZ (Lei et al., 2005; Sharma et al., 2006), administered systemically can also cause insulin dependent diabetes, but it lacks the autoimmune profile (Yu et al., 2000).

The potential problem with STZ is that its toxic effects are not restricted to pancreatic β-cells since it may cause renal injury (Valentovic et al., 2006), oxidative stress inflammation and endothelial dysfunction (Lei et al., 2005).

The destruction of pancreatic β-cells by both drugs is associated with a huge release of insulin which makes animals more susceptible to severe hypoglycemia that may be lethal. Thus, following treatment with either STZ or alloxan, animals are fed with glucose solution (5%) for 12–24 h. Afterwards, an increase of glucose levels is observed in comparison to control animals due to insulin deficiency. It is also reported that fasted animals are more susceptible to alloxan effects (Katsumata et al., 1992; Federiuk et al., 2004) and increased blood glucose in fed animals provides partial protection (Federiuk et al., 2004). In general, experimental protocols recommend that administration of either STZ or alloxan must be done in the fasting period (8–12 h), followed by addition of glucose solution to avoid hypoglycemia. Besides rats, dogs and mice, other animal species such as rabbits and monkeys have been employed to induce diabetes by these protocols, but rabbits and pigs are more resistant to STZ (Rees and Alcolado, 2005).

In general, 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. Glucose is measured by standard glucose-oxidase or dehydrogenase assays, mainly by means of commercial meters available everywhere. Insulin determination is available in experimental animals by different methodologies (radioimmunoassay—RIA or immunometric assays) (Esmaeili and Yazdanparast, 2004). In chronic experiments, A1c glycated hemoglobin can be also measured (Gupta et al., 2005b; Sathishsekhar and Subramanian, 2005; Sekar et al., 2005; Shirwaikar et al., 2005; Narendhirakkan et al., 2006; Santhakumari et al., 2006; Tanaka et al., 2006).

Table 2 displays a list of plants and/or their active compounds tested in diabetic animals (induced by either STZ or alloxan) published in the past 2 years.

It is necessary to reemphasize that natural products display several effects besides lowering blood glucose in these experimental models. In view of the lack of parallel studies of their toxicity, these models of diabetes induced by either alloxan or STZ are considered a screening step in the search for drugs for the treatment of diabetes (Kecskemeti et al., 2002).
Table 2: List of studied natural products with putative antidiabetic effects tested in either in vivo or in vitro models in the period of 2005–2006 (source: PUBMED, English language).

<table>
<thead>
<tr>
<th>Plant (family)</th>
<th>Material</th>
<th>Treatment</th>
<th>Drug-induced diabetes</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aegle marmelos</em> (Rutaceae)</td>
<td>EtOH/leaves</td>
<td>i.p., 14 d</td>
<td>STZ-rat</td>
<td>↓Glucose, ↓glycosylated hemoglobin, ↑C-peptide, ↑glucose tolerance, ↑glycogen, ↑insulin</td>
<td>Narendhirakannan et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>AE/seeds</td>
<td>p.o., 14 d</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Kesari et al. (2006)</td>
</tr>
<tr>
<td><em>Aloe vera</em> (Liliaceae)</td>
<td>EtOH, isolated compounds/leaves</td>
<td>p.o., 28 d</td>
<td>db/db mice</td>
<td>↓Glycosylated hemoglobin A1c</td>
<td>Tanaka et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Fruit-pulp</td>
<td>p.o., 10 d</td>
<td>Alloxan-rabbit</td>
<td>↓Glycosylated hemoglobin, ↑glucose tolerance</td>
<td>Gupta et al. (2005b)</td>
</tr>
<tr>
<td><em>Averrhoa bilimbi</em> (Oxalidaceae)</td>
<td>AE, BuOH, EtOAc, Hex/leaves</td>
<td>p.o., 14 d</td>
<td>STZ-rat</td>
<td>↓Glucose, ↓lipids</td>
<td>Tan et al. (2005)</td>
</tr>
<tr>
<td><em>Baccharis trimera</em> (Myrtaceae)</td>
<td>AE, EtOH, BuOH/leaves</td>
<td>p.o., 7 d</td>
<td>STZ-mice</td>
<td>↓Glucose</td>
<td>Oliveira et al. (2005)</td>
</tr>
<tr>
<td><em>Bryophyllum pinnatum</em> (Crassulaceae)</td>
<td>AE/leaves</td>
<td>p.o./i.p., AT</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Ojewole (2005a)</td>
</tr>
<tr>
<td><em>Camarium schweinfurthii</em> (Burseraceae)</td>
<td>MeOH/CH2Cl2/stem barks</td>
<td>p.o., 14 d</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Kamtchouing et al. (2005)</td>
</tr>
<tr>
<td><em>Chamaemelum nobile</em> (Asteraceae)</td>
<td>AE/leaves</td>
<td>p.o., 15 d</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Eddouks et al. (2005a)</td>
</tr>
<tr>
<td><em>Coscinium fenestratum</em> (Menispermaceae)</td>
<td>AAE/stem barks</td>
<td>p.o., 12 d</td>
<td>STZ-rat</td>
<td>↓Glucose, ↓glycosylated hemoglobin, ↓glycogen, ↓lipids, ↓oxidative stress</td>
<td>Shirwaikar et al. (2005)</td>
</tr>
<tr>
<td><em>Egyptian Morus alba</em> (Moraceae)</td>
<td>AAE, isolated compounds/stem barks</td>
<td>p.o., 10 d</td>
<td>STZ-rat</td>
<td>↓Glucose, ↓lipid peroxidation, ↑insulin</td>
<td>Singap et al. (2005)</td>
</tr>
<tr>
<td><em>Eugenia jambolana</em> (Asteraceae)</td>
<td>AE, EtOH, isolated compounds/stem barks</td>
<td>p.o., AT</td>
<td>STZ-rabbit</td>
<td>↓Glucose, ↓lipid, ↑glucose tolerance</td>
<td>Sharma et al. (2006); Ravi et al. (2005)</td>
</tr>
<tr>
<td><em>Hintonia standleyan</em> (Rubiaceae)</td>
<td>Garlic oil, isolated compounds</td>
<td>p.o., 21–112 d</td>
<td>STZ-rat</td>
<td>↑Glucose tolerance, ↓glucose, ↓oxidative stress</td>
<td>Liu et al. (2005, 2006); El-Demerdash et al. (2005)</td>
</tr>
<tr>
<td><em>Hyposis hemerocallidea</em> (Hypoxidaceae)</td>
<td>MeOH, isolated compounds/stem barks</td>
<td>p.o., AT</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Guerrero-Analco et al. (2005); Navarrete and Mata (2005)</td>
</tr>
<tr>
<td><em>Lycium barbarum</em> (Solanaeace)</td>
<td>Isolated compound/fruits</td>
<td>p.o., 21–26 d</td>
<td>STZ-rat</td>
<td>↓Glucose, ↓oxidative stress, ↑GLUT4, ↑insulin</td>
<td>Zhao et al. (2005b); Wu et al. (2006)</td>
</tr>
<tr>
<td><em>Mangifera indica</em> (Anacardiaceae)</td>
<td>AE/stem barks</td>
<td>i.p., AT</td>
<td>STZT-rat</td>
<td>↓Glucose</td>
<td>Ojewole (2005c)</td>
</tr>
<tr>
<td><em>Momordica charantia</em> (Cucurbitaceae)</td>
<td>MeOH, isolated compounds/gourd</td>
<td>p.o., AT</td>
<td>STZT-rat</td>
<td>↓Glucose, ↓glycosylated hemoglobin, ↓oxidative stress, ↑glycogen</td>
<td>Shetty et al. (2005); Harinantenaina et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>AE/leaves</td>
<td>p.o., 27–30 d</td>
<td>STZ-rat, alloxan-rat</td>
<td>↓Glucose, ↓glycosylated hemoglobin, ↓oxidative stress, ↑glycogen</td>
<td>Sekar et al. (2005); Reyes et al. (2006)</td>
</tr>
</tbody>
</table>
Numerous active principles may be present in the raw extract of *Terminalia superba* (Rajasekaran et al., 2005; Tanaka et al., 2006). These results suggest that the plant and highlight the need to further advance in their characterization. In line with this hypothesis, Tanaka et al. (2006) had isolated several compounds with antihyperglycemic properties from *Aloe vera* leaves (Tanaka et al., 2006). The identification of these products explains, in part, why these compounds present antioxidant, antihyperglycemic, antilypemic properties and even enhance the process of wound healing in diabetic and nondiabetic animals (Okyar et al., 2001; Sharma et al., 2003, 2006; Taniguchi et al., 2006). Due to the nonspecific action of compounds isolated from the plant and highlight the need to further advance in their characterization. In line with this hypothesis, Tanaka et al. (2006) had isolated several compounds with antihyperglycemic properties from *Aloe vera* leaves (Tanaka et al., 2006). The identification of these products explains, in part, why these compounds present antioxidant, antihyperglycemic, antilypemic properties and even enhance the process of wound healing in diabetic and nondiabetic animals (Okyar et al., 2001; Sharma et al., 2003, 2006; Pepato et al., 2005; Rajasekaran et al., 2005; Tanaka et al., 2006). Due to the nonspecific action of compounds isolated from extracts of natural products, some studies have aggregated additional in vitro protocols to the in vivo studies such as liver perfusion to evaluate glucose influx inhibition (Chung et al., 2006), gastrointestinal absorption methodologies and antiox-

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<th>Plant (family)</th>
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<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psidium guajava</em> Linn. (Myrtaceae)</td>
<td>AE/leaves</td>
<td>p.o., AT</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Ojewole (2005d)</td>
</tr>
<tr>
<td><em>Syzygium cumini</em> (synonym <em>Tamarindus indica</em>) (Caesalpiniaceae)</td>
<td>AE, EtOH, BuOH fraction/leaves and seeds</td>
<td>p.o., 7–14 d</td>
<td>STZ-mice, rat</td>
<td>↓Glucose</td>
<td>Maiti et al. (2005); Oliveira et al. (2005)</td>
</tr>
<tr>
<td><em>Taxus yunnanensis</em> (Taxaceae)</td>
<td>AE, MeOH, isolated compounds/woods</td>
<td>i.p., AT</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Banskota et al. (2006)</td>
</tr>
<tr>
<td><em>Terminalia chebula</em> (Combretaceae)</td>
<td>CH₂Cl₂ extract/seeds</td>
<td>p.o., AT</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Rao and Nammi (2006)</td>
</tr>
<tr>
<td><em>Terminalia superba</em> (Combretaceae)</td>
<td>MeOH, CH₂Cl₂/stem barks</td>
<td>p.o., 14 d</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Kamchouing et al. (2005)</td>
</tr>
<tr>
<td><em>Tremella mesenterica</em> (Combretaceae)</td>
<td>Isolated compounds/fruits</td>
<td>p.o., 14 d</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Dimo et al. (2006)</td>
</tr>
<tr>
<td><em>Triticum repens</em> (Brassicaceae)</td>
<td>AE/rhizomes</td>
<td>p.o., AT</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Lo et al. (2006)</td>
</tr>
<tr>
<td><em>Viscum album</em> (Loranthaceae)</td>
<td>AE, EtOH/whole plant</td>
<td>p.o., 7 d</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Eddouks et al. (2005b)</td>
</tr>
<tr>
<td><em>Zizyphus spina-christi</em> (Rhamnaceae)</td>
<td>BuOH, isolated compounds/leaves</td>
<td>p.o., AT</td>
<td>STZ-rat</td>
<td>↓Glucose</td>
<td>Orhan et al. (2005)</td>
</tr>
</tbody>
</table>


In addition, considering the diversity of active principles found in the crude plant extracts some studies also include further analysis of lipids (cholesterol, and triglycerides) as well as additional evaluation of the antioxidant properties of the product. This can be exemplified with studies with the fruit-pulp, seeds and leaves extracted from *Eugenia jambolona*, whose antidiabetic properties are largely recognized by folk medicine in several countries (Grover et al., 2002). Despite of the lack of dose standardization, plant environment selection and toxicological studies, different studies have confirmed that the ethanolic extracts of either the fruit-pulp or seeds of this plant present hypoglycemic, hypolipemic and antioxidant properties (Sharma et al., 2003; Pepato et al., 2005). These results suggest that numerous active principles may be present in the raw extract of the plant and highlight the need to further advance in their characterization. In line with this hypothesis, Tanaka et al. (2006) had isolated several compounds with antihyperglycemic properties from *Aloe vera* leaves (Tanaka et al., 2006). The identification of these products explains, in part, why these compounds present antioxidant, antihyperglycemic, antilypemic properties and even enhance the process of wound healing in diabetic and nondiabetic animals (Okyar et al., 2001; Sharma et al., 2003, 2006; Pepato et al., 2005; Rajasekaran et al., 2005; Tanaka et al., 2006). Due to the nonspecific action of compounds isolated from extracts of natural products, some studies have aggregated additional in vitro protocols to the in vivo studies such as liver perfusion to evaluate glucose influx inhibition (Chung et al., 2006), gastrointestinal absorption methodologies and antiox-
idant enzymatic systems, liver glycogen level, among others (Babu et al., 2006; Fadzelly et al., 2006; McAnuff-Harding et al., 2006). These protocols contribute to extend the analysis of the antidiabetic effects of a certain natural product. In this context, liver perfusion methodologies with the simultaneous measurement of glucose influx help to elucidate if the natural product exerts extra-pancreatic effects like metformin and glitazones. On the other hand, other studies suggest that inhibition of carbohydrate absorption may be linked to the antidiabetic properties of the natural product. Thus, inclusion of at least two different routes of treatment, for instance, i.p. and oral route (p.o.) can help in the analysis of the possible site of action of a studied natural product.

Vacor, dithizone (diphenylthiocarbazone), and 8-hydroxyquinolone may also cause experimental diabetes, but their use in research is restricted due to their level of toxicity (Clark et al., 1994).

2.2. Surgical models of diabetes

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, and primates (Choi et al., 2004; Liu et al., 2004; Rees and Alcolado, 2005; Masiello, 2006). Limitations to this technique include (1) high level of technical expertise and adequate surgical room environment, (2) major surgery and high risk of animal infection, (3) adequate post-operative analgesia and antibiotic administration, (4) supplementation with pancreatic enzymes to prevent malabsorption and (5) 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 (Masiello, 2006).

Choi et al. (2004) investigated the action or relative glucose uptake in various tissues of 90% pancreatectomized rats by using either hyperglycemic or euglycemic hyperinsulinemic clamp methodologies. This experimental design permits to evaluate if the compound has some effect upon both resistance to and secretion of insulin.

2.3. Genetic models of diabetes

2.3.1. Animal strains that spontaneously develop diabetes

These models permit the evaluation of the effect of a natural product in an animal without the interference of side effects induced by chemical drugs like alloxan and STZ reported above. Several recent publications summarized the major advances in this field (for review see Rees and Alcolado, 2005; Masiello, 2006) and at the website http://www.informatics.jax.org.

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. Thus, food intake is important in determining the severity of the diabetic phenotype and restriction of energy intake reduces both the obesity and hyperglycemia seen in this strain of mice. Another example is the spontaneously diabetic Goto-Kakizaki rat which is a genetic lean model of type 2 diabetes originating from selective breeding over many generations of glucose-intolerant nondiabetic Wistar rats (Chen and Wang, 2005). Regarding type 1 diabetes models, the NOD mouse typically presents hyperglycemia between 12 and 30 weeks of age, whereas in BB rats it occurs around 12 weeks of age. One great advantage of these models is that they can also be employed as model of atherosclerosis which represents the long-term complication of diabetes mellitus and tested against several natural products (Wu and Huan, 2007).

As it is shown in Fig. 2, the NOD mouse followed by the ZK rat are the most used to test natural products.

Other prone strains to type 1 diabetes mellitus include New Zealand white rabbit, Kreesbond dog, Chinese hamster and Celebes black ape. However, they have not been employed in studies to evaluate natural products to treat diabetes, except in preclinical trials of exenatide (incretin analog) (Rees and Alcolado, 2005).

2.3.2. Genetically engineered diabetic mice

In this case, rodents may be produced to over (transgenic) or under (knockout)-express proteins thought to play a key part in glucose metabolism. For a sound review on this topic see Masiello (2006) and Cleee and Attie (2006).

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 (Meiton, 2006).
2.4. Other models of type 2 diabetes to evaluate the reduction of pancreatic β-cell mass

The importance of the progressive loss of pancreatic β-cell reduction in the course of type 2 diabetes has been the focus of therapeutic targets in the development of novel and potential drugs acting by enhancing pancreatic β-cell growth and/or survival (Hansotia and Drucker, 2005; Masiello, 2006). Experimental reduction of pancreatic β-cell mass by either surgical or chemical means has been reported above, but these models are limited due to the variety amount of residual pancreatic β-cell content. On the other hand, experimental studies carried out predominantly in rodents have demonstrated that incretins, IGFs, hepatocyte growth factor, pituitary adenylate cyclase-activating polypeptide are capable of enhancing pancreatic β-cell mass in the field of experimental diabetes (Stoffers et al., 2003). Hence, prolonged administration of these agents may expand pancreatic β-cell mass, leading to increased insulin secretion and improved glycemic control (Hansotia and Drucker, 2005).

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 (Holemans et al., 2003). 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 (Simmons et al., 2001). This has been achieved by several means, including bilateral uterine artery ligation (Simmons et al., 2001; Boloker et al., 2002), at 19 days of gestation, i.e. 3 days before term. The diabeticogenic 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 (Reusens and Remacle, 2001).

Finally, animal models with increased pancreatic β-cell apoptosis have also been developed, besides those cited in Section 2.3 (Matveyenko and Butler, 2006).

3. In vitro studies

3.1. In vitro studies on insulin secretion

Conventional antidiabetic agents can affect several pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs as well as nutrient absorption. Recently, incretins (Hansotia and Drucker, 2005), and transcription factors such as peroxisome proliferator-activated receptors—PPAR (Rosenson, 2007) are targets of modern therapy. Insulin receptor, glucose transporters, however, has not been yet the focus of antidiabetic therapy. Although few studies using natural products have been published (Iwashima et al., 2001; Storling et al., 2005; Zhao et al., 2005a), these methodologies may serve as complementary tools to explore findings obtained in in vivo models.

3.1.1. Studies using isolated pancreatic islet cell lines

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) (Affourtit and Brand, 2006). The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage-dependent Ca2+ channels. This results in elevation of the intracellular Ca2+ concentration which triggers insulin secretion (Ashcroft and Rorsman, 2004). In type 2 diabetes, pancreatic β-cells exhibit atypical ion channel activity and an abnormal pattern of insulin secretion (Ashcroft and Rorsman, 2004). 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 (Zhao et al., 2005a; Storling et al., 2005). Afterwards, the experimental protocol is assayed.

3.1.2. 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 cells (Poitout et al., 1996). 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 (Poitout et al., 1996).

3.2. 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 (Lelliott and Vidal-Puig, 2004). 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 (Karalee et al., 2001) and rat L6 muscle engineered to over-express GLUT4 (Maddux et al., 2001) and may be employed as tools to evaluate the effects of natural products upon glucose uptake.

4. Models of diabetes accelerated atherosclerosis

Accelerated cardiovascular disease is a leading cause of both morbidity and mortality in diabetic patients (Wu and Huan, 2007). Aggressive therapy of dyslipidemia is necessary, since the risk of myocardial infarction is the same as in nondiabetic patients with previous myocardial infarction (Haffner et al., 1998). Currently, rats and mice are the most widely used mod-
els do not study diabetes and atherosclerosis. Albeit, diabetic mice do not exhibit a high degree of atherosclerosis unless hyperglycemia is associated with severe hyperlipidemia, a fat diet is also present in these protocols. Models of diabetic nephropathy, a microvascular complication have also been developed (Breyer et al., 2005; Wu and Huan, 2007). For a review of these models and other informations see the website: www.mmmpc.org.

5. Potential effects of novel antidiabetic drugs

At present no drug is able to arrest the progressive loss of pancreatic β-cells which occurs in type 2 diabetes mellitus. According to the United Kingdom Prospective Diabetes Study—UKPDS results, at the time of type 2 diabetes mellitus diagnoses, 50% of pancreatic β-cell function had already been lost (Kan, 2001). Thus, the efficacy and side effect of marketed oral antidiabetic drugs still need to be optimized. The recent introduction in the market of incretin analogs opened a new field to evaluate drugs with putative properties that may cause both proliferation and maturation of human pancreatic β-cells (Hansotia and Drucker, 2005).

Currently, 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.

6. Concluding remarks

In spite of the worldwide use of herbs and medicinal plants, the effective treatment of diabetes with phytochemicals has not yet been validated with scientific criteria which may support their substitution for the current therapy. Although some studies have been published with raw natural products they have neither shed light on the mechanisms of action of these products nor have they shown a potential to be employed as new therapeutic drugs. This implies that several models are necessary to called for, in addition to the demonstration that a putative natural product exerts antihyperglycemic activity. Thus, by focusing on other targets of pancreatic islet cell dysfunction, new models may help to elucidate effects of medicinal plants employed in the treatment of diabetes mellitus.

References


