MEDICINAL PLANTS: A METHODOLOGY FOR STUDYING THEIR ANTI-DIABETIC ACTIVITY

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ABSTRACT

This work is a review on the study methods of the antidiabetic activity of medicinal plants. Indeed, this discipline is not standardized and techniques used to assess this activity are very varied. The choices of the plant and the extract to be tested are major parameters before going to the model of diabetes. The latter is induced into animals by various techniques including the injection of chemicals such as streptozotocin which damages the pancreatic β cells. Hence, diabetes develops spontaneously in certain animal species. Works realized on animals (preclinical) would permit to research on human being (clinical).

KEYWORDS: Diabetes, Medicinal Plants, Antidiabetic Plants, Streptozotocin, Alloxan

INTRODUCTION

Methods for studying medicinal plants anti-diabetic activity are diverse. Some are processed in vivo on animals (pre-clinical studies) and others on diabetic patients (clinical studies) or in vitro on cellular cultures. Before launching a study, most important topics to settle are: diabetes model, treatment route, plant extracts preparation type, as well as anti-diabetic evaluation parameters.

DEFINITION OF EXPERIMENTAL DIABETES

Experimental diabetes consists in producing on animals a similar state to diabetes mellitus, with the intention of better understanding human diabetes and finding new therapies (Wright et al., 1980).

According to the U.S. National Research Council Committee on Animal Models for Research and Aging, animal models for biomedical research should have the following requirements: a normative biology or behavior that can be studied, an induced or spontaneous pathological process that can be investigated, and phenomena in one or more aspects that can be compared to humans or other animal species (Eddooks et al., 2012).

Based on these guidelines, animal models used for biomedical research can be classified into five groups:

- Spontaneous models where disease or its conditions are produced spontaneously by animals as humans,
- Experimental models where disease or its conditions are produced experimentally,
- Genetically modified models where disease or its conditions are induced through chemistry, surgery or genetic manipulations,
- Negative models including animals resistant to one particular disease or condition,
- Orphan models including animal models with unknown equivalent disease on humans (Chatzigeorgiou et al., 2009).
These last years, studies undertaken to settle appropriate animal diabetes models, specifically type 2 diabetes (with no associated obesity), led to different model types mainly with rats. Using such diabetes models confirm the idea that abnormalities of insulin secretion and insulin sensitivity should be secondary to a more or less marked reduction of β cells population (Etuk, 2010).

**ANIMAL MODELS OF DIABETES MELLITUS**

Experimental diabetes can be induced in animals through injection of diabetogenic substances (Crouch et al., 1978) or spontaneously through a hypercaloric diet or stress (Vogel et Vogel, 1997).

Experimental diabetes through pancreatectomy is not used anymore because of technical difficulties and also because it causes ablation of insulin secretion, the purpose of such study. It also causes ablation of other hormones like glucagon (Dupin, 1992).

**SPONTANEOUS DIABETES**

Many animal species have a strong predisposition to diabetes mellitus like certain mice, rats, hamsters, guinea pigs, small pigs, monkeys and others (Ganong, 2005).

Diabetes model is selected according to the investigated pathology. Certain animals develop a diabetes type similar to type 2 or type 1. In certain circumstances induced pathology is only a part of diabetes mellitus, an insulin resistance, an auto-immune reaction towards β cells, a retinopathy...

Selecting diabetes model is though a crucial topic to confirm tested extracts or molecules effects.

**Type 1: Diabetes**

Five spontaneous diabetes animal models are mainly selected to study auto-immune diabetes: NOD mice, BB, LETL, PDK and LEW-DID rats. NOD mice and BB rats are the most used (Chatzigeorgiou et al., 2009).

**Bio-Breeding Rats (BB Rats)**

BB rats are spontaneous diabetes models associated to insulin deficiency as well as to insulinite due to auto-immune destruction of pancreatic β cells. It can be more severe than with streptozotocin induced rats diabetic (Horowitz et Samson, 2004). Both male and female BB rats develop type 1 diabetes after exposure to stress (Vogel et Vogel, 1997; Mordeset al., 2004).

**Non Obese Diabetic Mice (NOD Mice)**

NOD mice are a model for studying type 1 diabetes. It is a mice type of consanguine origin. Ninety to one hundred eighty days after birth, a majority develops a spontaneous diabetes similar to human type 1 diabetes. Their particularity is having a specific type of CMH II (IA g7 type) and developing spontaneously the disease (Chatzigeorgiou et al., 2009).

Many characteristics of human type 1 diabetes and BB rats’ diabetes match in this model: auto-immune destruction of β cells from pancreatic islets, T lymphocytes abnormal activity, sensibility of disease to immunodepressant treatments, and a genetic constituent of disease located in CMH (Cavanet et al., 1992; Chatzigeorgiou et al., 2009).

**Type 2: Diabetes**

Type 2 diabetes mellitus is a very complex and heterogenic disease. Many species can develop diabetes similar to this pathology. Most often, these animals are obese rats and mice species (*Psammomysobesus*, *ob/ob* mice, *db/db* mice and...
Zuckerfa/fa rat) or non-obese rats and mice species (Goto-Kakizaki rats and non-obese mutant C57BL/6 Akita mice) (Chatzigeorgiou et al., 2009).

Psammomysobesus Rats (PO Rats)

PO rats belong to the Gerbillidae family and are also known as “sand rats”. They live in North Africa and Middle East countries deserts (Vogel et Vogel, 1997). Their diet mainly consists in leaves and stems from Chenopodiaceae family plants, very rich in mineral salts, water and fibers (Dupin, 1992).

In its natural environment, PO rats eat low calories salty plants. Under standard laboratory diet, 40% of animals become obese and develop type 2 diabetes after two months. The other 60% do not develop diabetes but remain obese with high plasmatic insulin level (Shafrir et Renold, 2001).

Sand rats react to feeding growth inducing caloric overload by increasing body weight. This is due to adipocytes or fatty cells increasing size. Sand rats also react through hyperinsulinemia and intolerance to glucose at different degrees. Diet effect on diabetes installation is reversible. On the other hand, after 6 months diet, some animals with diabetes have a major weight and plasmatic insulin level drop as well as an increasing plasmatic glucose level. These sand rats develop dependant insulin diabetes, the disease last step (Marquié et al., 1997; Shafrir et al., 2006).

ob/ob and db/db Mice

The two species have a genetic mutation inducing obese type 2 diabetes with autosomal recessive transmission. The affection physiopathology is a leptin synthesis (or its function) defect.

For ob/ob mice (relabeled as Lepob) it consists in a mutation on chromosome 6 in C57BL/6J mice strain. Mutation is on ob gene encoding leptin synthesis, what induces animal overweight and obesity leading to insulin resistance (Srinivasan et Ramarao, 2007).

For db/db mice (relabeled as leprdb) it consists in a mutation on chromosome 4 in C57BL/KsJ mice strain, precisely on db gene encoding leptin receptors synthesis. Animals are polyphagic, obese and get insulin resistance (Chatzigeorgiou et al., 2009).

Goto-Kakizaki Rats (GK Rats)

GK rats are a model of polygenic diabetes produced by Goto and collaborators (Goto et al., 1975). It was done through selective inbreeding of Wistar rats with abnormal glucose tolerance.

Its characteristics are: no obesity, moderate but stable fasting hyperglycemia, hypoinsulinemia, normolipidemia and impaired glucose tolerance. Pathophysiologies are recorded after two weeks of age with severe complications (Portha, 2003).

On adults, β cells mass have a 60% reduction with an insulin stock dropping in pancreatic cells. Besides, insulin resistance is recorded in liver, skeleton, muscles and adipose tissues (Chatzigeorgiou et al., 2009; Srinivasan et Ramarao, 2007).

CHEMICALLY INDUCED DIABETES

Streptozotocin Induced Diabetes

Diabetes mellitus can be induced in animals through different techniques: STZ injections is one of them and is widely used (Szkudelski, 2001). STZ is a glucosamine-nitrosourea (figure 1) (Anderson et al., 1974; Povoskiet al., 1993)
leading to a selective cytotoxic effect on Langerhans islets β cells (Anderson et al., 1974; Robbins et al., 1980; Crouch et al., 1978).

How the diabetogenic agent works is still not very well known. However, former studies have demonstrated how it acts on Langerhans islets by reducing β cells mass and as a consequence by inducing an insulinopaenia, a characteristic of chronic or transitory hyperglycemia (Aughsteen, 2000; Szkudelski, 2001; Chen et Ianuzzo, 1981).

Glucose constituting STZ molecules allows it to penetrate into pancreatic β cells through GLUT2 glucose transporters. Inside the cell, STZ breaks down into reactive oxygen species causing a DNA alkylation and defragmentation. This activates poly (ADP-ribose) polymerase, key enzyme for DNA repairing. The reaction consumes NAD and ATP as cofactors leading to their depletion and to β cells necrosis (Szkudelski, 2001).

STZ causes an alteration of carbohydrate, lipid and protein metabolism due to insulin loss (Szkudelski, 2001; Szkudelski et Szkudelska, 2002; Junod et al., 1969). However, former studies revealed toxin indirect effect in signaling insulin. Steady hyperglycemia causes insulin resistance as a result of the hormone receptor autophosphorylation loss (Kadowaki et al., 1984). It was demonstrated that it activates protein kinase C expression, a protein responsible of insulin receptor dephosphorylation (Davidoff et al., 2004). Moreover, STZ injection causes weight loss (Junod et al., 1969; Chen et Ianuzzo, 1981).

For better understanding STZ pathogenic mechanism, many works demonstrated that the product reduces cell antioxidant defense, particularly a superoxide dismutase activity inhibition (Robbins et al., 1980; Gandy et al., 1982; Crouch et al., 1978; Rajasekaran et al., 2005).

STZ doses are adjustable according to route of administration, animal and above all expected pathology (Junod et al., 1969; Chen et Ianuzzo, 1981). For example, in preliminary essays, when animals’ vitality is important, only small STZ doses are injected (less or equal to 60mg/kg per IV) (Jarrinet et al., 2002).

**ALLOXAN INDUCED DIABETES**

Alloxan, the 2,4,5,6-tetraoxypyrimidine, is an oxygenated pyrimidine. The molecule is prepared through oxidation of uric acid by the action of nitric acid (Szkudelski, 2001).
cells. In cytosol, alloxan is reduced into dialuric acid. The reduction is caused by several agents such as reduced glutathion, cysteine, ascorbic acid and SH protein groups. Alloxan is related with two thiol groups from glucokinase active site creating a disulfide bond and deactivating enzyme (Lenzenet et al., 1988).

Created dialuric acid is re-oxidized into alloxan and generates reactive oxygen species, an active Fenton reaction (Skudelski, 2001; AnkuretShahjad, 2012).

On the other hand, reactive oxygen species attack DNA and induce its defragmentation. Mechanism is similar to streptozotocin (AnkuretShahjad, 2012).


PREPARING PLANT MATERIAL

In order to evaluate eventual plants anti-diabetic effects, scientists prefer referring to Mother Nature by studying several topics such as part of the plant to be used, season to collect plants, preparation, traditional use, treatment duration, etc.

Preparations often remain as they were: basically aqueous brut extracts, plain powder, juice, fresh or dried plant, etc. Extracts are prepared through maceration, infusion or decoction.

First required step before studying is a botanic identification of plant species by several botanists. We cannot expect taking profit of the whole natural drug efficiency if the plant under research is not the right one or if the quality is bad (Harbone, 1998). To take the best from medicinal plants, it is indeed convenient to make sure that herbs and their derivates are from excellent quality. This requires that they are cultivated in good conditions, correctly dried up, well preserved and used before their expiration date. Using bad quality plants often means losing time and money (Larousse, 2001).

Preserving Mother Nature, we start investigation on plants activity with brut extracts where in majority of cases water and in second position ethanol are used (Harbone, 1998; Khatibi, 2011). With the purpose of separating active molecules, we proceed then with fractionation techniques (Bruneton, 1999).

PLANT EXTRACTS ADMINISTRATION ROUTE

In order to scientifically evaluate prepared extracts, they must be dried before extraction. It allows their good conservation, a control of administrated doses as well as concentration of active principles (Mathieu etFonteneau, 2008).

While administrating to animals, extracts are re-solubilized in a proper solvent. For lipophilic extracts, detergents as tween 80 or gum tragacanth can be used. The reconstituted extract must be very well homogenized or even solubilized (Sinead et al., 2004).

For several reasons, oral route is often selected as administration route. On one hand, as we refer to ethno-pharmacological principals, people generally use anti-diabetic plants by drinking tisanes made out of them or eating the complete plant. It is subsequently the closest route to what people do in reality (Eddoukset al., 2002). On the other hand, it is a physiological administration route offering a certain number of efficiency and convenience criteria. Besides all, it does not need any particular material. On a pharmacological point of view, oral route is the most frequently used (70 to 80% of drugs are administrated per os). It is generally a very well accepted route among patients (Bourin et Jolliet, 1999).
Despite advantages, oral route may cause a reduction of active molecules activity due to degradation by hepatic enzymes (Touitou, 2007). Active molecules may be destructed by digestive saps and certain components may irritate digestive tube. As a consequence, parenteral route becomes more advantageous than per os (Lüllmann et al., 1998; Garau et al., 2003; Wang et al., 2003).

EVALUATION OF ANTI-DIABETIC ACTIVITY

Research on animals, called preclinical research, lasts years and starts with studies on long and short term toxicity.

Pharmacological studies to evaluate anti-diabetic effects of powder or extract are first conducted on healthy animals and on animals with diabetes. In vitro tests are also possible on cell cultures, but cannot replace studies on animals: they are better records of drug effects on organism (Cohen & Jacquot, 2011).

To evaluate anti-diabetic activity, several physiological parameters are studied. Body weight variations are important parameters besides biochemical parameters: they vary according to animal models and research steps. Composition and structure of tissues are also investigated (Wright et al., 1980).

Transition to clinical studies on humans is decided if drug development seems to be interesting after former toxicological and pharmacological studies on animals. Clinical researches have a legal procedure with four steps therapeutic tests (Kintz, 1998).

In step 1, research is conducted on healthy volunteers to control effects recorded on animals and settle a dose-effect relation. In step 2, tests are conducted on diabetic patients. In case of substance having real efficiency and no secondary effects, next step is engaged. In step 3, therapeutic action of new substance is compared to standard drug and must be conducted on a greater number of patients. First steps allow drug marketing and step 4 consists in drug and secondary effects follow-up (Lüllmann et al., 1998; Touitou, 2007).

CONCLUSIONS

We can conclude, from this review, that before testing the antidiabetic activity of a plant, be sure the botanical species and try to get more information on its traditional use as a remedy. From now, we can fix the extraction protocol and the way of administration. As far as the model of diabetes is concerned, we choose the one whose physiopathology induced is closes to that of man.

REFERENCES


