ANTI-DIABETIC ACTIVITY: METHODS FOR EVALUATION

Abstract

Diabetes mellitus is a chronic condition characterized by insufficient insulin production or ineffective insulin utilization, resulting in elevated blood glucose levels. This review outlines the various types of diabetes, including type 1, type 2, and gestational diabetes, and their potential complications, such as cardiovascular diseases, neuropathy, and nephropathy. It explores the mechanisms of action and examples of common anti-diabetic medications, including biguanides, sulfonylureas, meglitinides, thiazolidinediones, alpha-glucosidase inhibitors, DPP-4 inhibitors, and SGLT2 inhibitors. The review further details the evaluation methods for anti-diabetic activity, encompassing both in-vitro assays, such as α-amylase and α-glucosidase inhibition assays, and in-vivo models, like streptozotocin-induced and high-fat diet-induced diabetes in rodents. These methodologies are crucial for assessing insulin sensitivity, glucose metabolism, and other diabetes-related markers, providing insights into the efficacy and safety of potential anti-diabetic therapies.

Keywords: Diabetes mellitus, insulin, anti-diabetic medications, biguanides, sulfonylureas, thiazolidinediones, α-amylase inhibition assay, α-glucosidase inhibition assay, streptozotocin-induced diabetes, high-fat diet-induced diabetes.

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I. INTRODUCTION

Diabetes mellitus is a chronic disease that occurs when the pancreas does not produce sufficient insulin or when the body cannot use it effectively, thereby leading to an increased blood glucose concentration. This can lead to high blood sugar levels, which can cause serious damage to the body over time, particularly to the nerves and blood vessels.

There are different types of diabetes, including type-1, type-2, and gestational diabetes. Type 1 diabetes is characterized by deficient insulin production and requires daily administration of insulin. Type-2 diabetes affects how the body uses sugar for energy and can often be prevented through lifestyle changes. Gestational diabetes occurs during pregnancy and can increase the risk of complications for both the mother and the baby. Over time, diabetes can damage blood vessels in the heart, eyes, kidneys and nerves. People with diabetes have a higher risk of health problems including heart attack, stroke and kidney failure. Diabetes can cause permanent vision loss by damaging blood vessels in the eyes. Many people with diabetes develop problems with their feet from nerve damage and poor blood flow. This can cause foot ulcers and may lead to amputation.

II. ANTI-DIABETIC MEDICATION

Anti-diabetic medications are drugs used to manage and treat diabetes mellitus; a condition characterized by high blood sugar levels. These medications belong to several classes, each with its own mechanism of action. Here are some common classes of anti-diabetic drugs along with their mechanisms of action and examples:

- **Biguanides:** Biguanides, such as metformin, primarily work by decreasing the production of glucose in the liver and improving insulin sensitivity in peripheral tissues, thus reducing insulin resistance. Example: Metformin (brand names: Glucophage, Glumetza).
- **Sulfonylureas**: Sulfonylureas stimulate insulin secretion from pancreatic beta cells by binding to ATP-sensitive potassium channels on the cell membrane, leading to depolarization and subsequent insulin release. Examples: Glibenclamide (glyburide), glipizide, glimepiride.
- **Meglitinides (Glinides):** Meglitinides work similarly to sulfonylureas by stimulating insulin secretion from pancreatic beta cells, but they have a faster onset and shorter duration of action. Examples: Repaglinide, nateglinide.
- **Thiazolidinediones (TZDs):** Thiazolidinediones primarily work by improving insulin sensitivity in peripheral tissues, such as muscle and fat cells, by activating peroxisome proliferator-activated receptor gamma (PPAR-γ). Examples: Pioglitazone, rosiglitazone.
- **Alpha-glucosidase inhibitors**: Alpha-glucosidase inhibitors slow down the absorption of carbohydrates from the digestive tract by inhibiting the enzymes responsible for breaking down complex carbohydrates into simple sugars. Examples: Acarbose, miglitol.
- **Dipeptidyl Peptidase-4 (DPP-4) Inhibitors**: DPP-4 inhibitors enhance the action of incretin hormones, such as GLP-1 (glucagon-like peptide-1), which stimulate insulin secretion and inhibit glucagon release in a glucose-dependent manner. Examples: Sitagliptin, saxagliptin, linagliptin.

- **Sodium-Glucose Co-Transporter 2 (SGLT2) Inhibitors**: SGLT2 inhibitors block the reabsorption of glucose in the kidneys, leading to increased urinary glucose excretion and lowering blood sugar levels. Examples: Canagliflozin, dapagliflozin, empagliflozin.

These are a few of the anti-diabetic drug classes that are frequently prescribed; each has a unique mode of action that aims to regulate blood sugar levels in people with diabetes mellitus. It's crucial to remember that the type of diabetes, its severity, coexisting medical disorders, and the unique characteristics of each patient all influence the prescription selection. For individualized guidance and care, always seek the assistance of a healthcare professional.

III. EVALUATION OF ANTI-DIABETIC ACTIVITY

A common method of measuring anti-diabetic action is to assess a range of markers pertaining to insulin sensitivity, glucose metabolism, and other diabetes-related variables. Several parameters that are frequently evaluated include:

- **Blood glucose levels**: Probably the most accurate indicator of anti-diabetic action. A major objective in the therapy of diabetes is improved glycemic control, which is shown by lowering blood glucose levels.
- **Insulin Sensitivity**: Measuring a cell's reaction to insulin can reveal information about how well anti-diabetic medications work. Methods like the homeostasis model assessment of insulin resistance (HOMA-IR) and the hyperinsulinemic-euglycemic clamp can be used to measure this.
- **Glycated Hemoglobin (HbA1c):** HbA1c reflects average blood glucose levels over the past two to three months and is an important marker for long-term glycemic control. Reductions in HbA1c indicate improved diabetes management.
- **Oral glucose Tolerance Test (OGTT)**: Following a standardized glucose load, the OGTT gauges how well the body's cells can absorb glucose. Increased metabolic function is indicated by improvements in glucose tolerance.
- **Serum Insulin Levels**: Monitoring changes in circulating insulin levels can provide information about insulin secretion and pancreatic beta-cell function.
- **Lipid Profile:** Diabetes often coexists with dyslipidemia, characterized by abnormal levels of lipids such as cholesterol and triglycerides. Improvements in lipid profile parameters may accompany effective anti-diabetic interventions.
- **Body Weight:** A lot of anti-diabetic drugs have an impact on body weight. Depending on how the medication works, one may have weight loss or stability.
- **Oxidative Stress Markers**: Since diabetes is linked to higher levels of oxidative stress, monitoring markers like malondialdehyde (MDA) or superoxide dismutase (SOD) activity can help determine the degree of oxidative damage and how well anti-diabetic treatments work to lessen it.
- **Inflammatory Markers**: Diabetes and its consequences are partly caused by chronic inflammation. One can examine markers like interleukin-6 (IL-6) and C-reactive protein (CRP) to evaluate the state of inflammation and therapy response.
- **Renal Function Markers**: Nephropathy, or damage to the kidneys, can result from diabetes. Tracking indicators such as blood urea nitrogen (BUN), urine albumin, and

serum creatinine can give light on renal function and the development of diabetic kidney disease.

- **Diabetic neuropathy and microvascular problems**: Measuring the parameters associated with diabetic neuropathy (damage to the nerves) and microvascular complications (such as retinopathy) might assist in determining how anti-diabetic medications affect diabetic complications more broadly. Preclinical and clinical trials frequently assess these indicators to ascertain the safety and effectiveness of possible anti-diabetic medications.

***In-vitro* assessment of anti-diabetic activity:**

α- amylase inhibition assay:

One gram of starch was dissolved in 100 ml of 20 mM phosphate buffer (pH 6.9) and 6.7 mM sodium chloride to obtain a starch solution (1% w/v). A solution of 27.5 mg α-amylase was prepared in 100 ml of phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. Test drugs (25, 50, 100, 200, 400 mg/ml) were added to 100 ml of α-amylase and incubated at 37°C for 20 min. A 10% starch solution was added in100 ml of the reaction mixture. It was incubated at 37°C for 10 minutes. Further, 3,5-di-nitrosalicylic acid (1 g), 30 g of sodium potassium tartrate and 20 ml of 2N-sodium hydroxide were added to a boiling solution for 5 minutes. Volume was made up to 100 ml. To measure absorbance, 2.2 ml of water was used to dilute the reaction mixture. Blank tubes were prepared for each concentration by replacing the enzyme solution with 200 µl of distilled water. The α- amylase inhibitor (acarbose) was used as standard. An enzyme-free control was prepared in the same manner, but without the test drug. Following the same procedure, the experiments were carried out for the third time α-amylase enzyme inhibition percentage:

$$I\ \% = (Ac - As)/Ac \times 100$$

Where, I % - inhibition percentage; Ac - absorbance value for the control and As - absorbance value for sample. The value is calculated as the average standard error of three repetitions.

α-glucosidase inhibition assay:

Inhibition of α-glucosidase activity was determined using yeast α- glucosidase and p-nitrophenyl-α-D-glucopyranoside. Acarbose and extract (100 μl of 25, 50, 100, 200, 400 mg/ml) was added to 50 μl of α-glucosidase (1 U/ml) prepared in 0.1 M phosphate buffer (pH 6.9), and 250 μl of 0.1 M phosphate buffer to get 0.5 to 5.0 mg/ml final concentration. The mixture was pre-incubated at 37°C for 20 min. After pre-incubation, 10 μl of 10 mM p-nitrophenyl- α-D-glucopyranoside prepared in 0.1 M phosphate buffer (pH 6.9) was added, and incubated at 37°C for 30 min. The reactions were stopped by adding 650 μl of 1 M sodium carbonate, and the absorbance was measured in a spectrophotometer (Shimadzu UV-1800) at 405 nm. The α-glucosidase inhibitor (acarbose) was used as standard. The percentage of inhibition of α-glucosidase was determined by given formula:

$$I\ \% = (Ac - As)/Ac \times 100$$

Where, I % - inhibition percentage; Ac - absorbance value for the control and As - absorbance value for sample. Values are represented as mean ± standard error mean of three values.

In-vivo assessment of anti-diabetic activity:

Evaluation of Streptozotocin (STZ) induced diabetes:

The streptozotocin (STZ)-induced diabetic evaluation method is a widely used experimental model to induce diabetes in rodents, particularly rats and mice. Diabetes is induced by administering STZ, a naturally occurring chemical compound, usually through intraperitoneal injection (typically dissolving in a citrate buffer to enhance its solubility and minimize irritation at the injection site). STZ selectively destroys pancreatic beta cells, leading to insulin deficiency and hyperglycemia, mimicking aspects of type 1 diabetes mellitus.

After STZ administration, blood glucose levels are monitored regularly using glucometers or by collecting blood samples from the tail vein or other suitable sites. Elevated blood glucose levels indicate the successful induction of diabetes. Diabetes induction is confirmed by observing sustained hyperglycemia (usually defined as blood glucose levels above a certain threshold, e.g., 200 mg/dL) in multiple consecutive measurements, typically over a period of several days.

Once diabetes is confirmed, researchers can evaluate the effects of various interventions, such as potential antidiabetic drugs, dietary modifications, or lifestyle interventions, on blood glucose levels, insulin sensitivity, and other relevant parameters and are analyzed statistically to determine the efficacy of the interventions in ameliorating diabetes-related symptoms and complications.

Overall, the STZ-induced diabetic evaluation method provides a valuable tool for studying the pathophysiology of diabetes and evaluating potential therapeutic strategies for its management. However, it's important to recognize that this model primarily reflects aspects of type 1 diabetes and may not fully capture the complexity of type 2 diabetes mellitus.

Evaluation of high-fat diet (HFD) induced diabetes:

The high-fat diet (HFD)-induced diabetic evaluation method is an experimental model used to induce type 2 diabetes mellitus (T2DM) in rodents, particularly mice and rats. Animals are fed a high-fat diet containing a significantly higher percentage of calories from fat compared to a standard rodent chow diet. The exact composition of the high-fat diet can vary, but it typically contains around 45-60% of calories from fat, whereas standard chow diets usually contain around 10-20% fat. Animals are fed the high-fat diet for an extended period, often several weeks to several months, to induce metabolic disturbances characteristic of T2DM, such as insulin resistance and obesity.

Throughout the study, body weight and food intake of the animals are monitored regularly to assess the development of obesity and metabolic changes induced by the high-fat diet. Blood glucose levels are monitored regularly using glucometers or by collecting blood samples from the tail vein or other suitable sites. Additionally, insulin levels may be measured to assess insulin resistance. Oral glucose tolerance tests (OGTT) or intraperitoneal glucose tolerance tests (IPGTT) may be performed to assess glucose tolerance and insulin sensitivity. These tests involve administering a glucose solution and monitoring blood glucose levels at specific time points. Insulin sensitivity can be assessed using techniques such as the insulin tolerance test (ITT) or the hyperinsulinemic-euglycemic clamp technique. These tests measure the response of the animals to insulin administration.

Tissue samples from organs such as the liver, adipose tissue, and pancreas may be collected and subjected to histological analysis to assess changes associated with insulin resistance, inflammation, and lipid accumulation.

Overall, the HFD-induced diabetic evaluation method provides a valuable tool for studying the pathophysiology of T2DM and evaluating potential therapeutic strategies for its prevention and treatment, particularly those targeting insulin resistance and obesity.

IV.CONCLUSION

Diabetes mellitus, a chronic disease characterized by high blood glucose levels, leads to significant health complications if not managed effectively. Various types of diabetes, including type 1, type 2, and gestational diabetes, require different therapeutic approaches. Anti-diabetic medications play a crucial role in managing this condition, with each class targeting different mechanisms to regulate blood sugar levels.

To evaluate the efficacy of anti-diabetic treatments, both in-vitro and in-vivo methods are employed. In-vitro assays, such as the α-amylase and α-glucosidase inhibition assays, help identify potential anti-diabetic agents by measuring their ability to inhibit enzymes involved in carbohydrate metabolism. In-vivo models, including streptozotocin (STZ)-induced and high-fat diet (HFD)-induced diabetes in rodents, are essential for studying the pathophysiology of diabetes and testing new therapeutic strategies.

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