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A REVIEW ON PHARMACOLOGICAL SCREENING FOR ANTI-DIABETIC DRUGS

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ABSTRACT
One of the most prevalent endocrine metabolic disorders, diabetes has an adverse effect on patients' health, quality of life, and life expectancy as well as the healthcare system. A clinical illness known as diabetes insipidus (DI) is defined by the progression of Unusually high levels of urine (diabetes) that are hypotonic, watery, and tasteless due to dissolved solutes (i.e., insipid). They are a subset of genetic or acquired polyuria and polydipsia illnesses. Hypotonic polyuria is the result of insufficient arginine vasopressin (AVP), antidiuretic hormone (ADH), or renal response to AVP and underlying/compensatory polydipsia. Hyperglycemia and abnormalities in the metabolisms of carbohydrates, proteins, and fats are symptoms of diabetes mellitus (DM), which is brought on by a complete or partial deficiency of the hormone insulin. Few of the secondary metabolites that have been traditionally utilized to treat diabetes around the world have been shown effective by scientific standards. The need for alternative strategies for the prevention and treatment of diabetes is growing rapidly as type-2 diabetes is accomplishing epidemic status in our society. The development for novel bioactive compounds with antidiabetic properties to deal with the disease condition is still in progress. In the present review, we have attempted to bring together all the reported models for screening antidiabetic drugs using In vivo and In vitro models And the various in vitro techniques which include Inhibition of α-Amylase, α-Glucosidase Activity, and glucose uptake assay. For the improvement of our knowledge, a comprehensive understanding of diabetogenic, and the development of novel treatments, experimental induction of diabetes in animal models, and in vitro approaches are crucial. The use of in vitro procedures and animal models is a Component necessary for creating a novel diabetic medication.

KEYWORDS: Diabetes Mellitus, Anti-diabetic screening, Diabetes, Streptozocin.

INTRODUCTION
Diabetes is a dangerous, chronic condition that develops when the pancreas either generates insufficient amounts of the hormone insulin (which controls blood glucose) or when the body struggles to utilize the insulin that is produced. Uncontrolled diabetes's major side effect, elevated blood glucose, can cause catastrophic long-term damage to the heart, blood vessels, eyes, kidneys, and nerves. Diabetes affects more than 400 million people worldwide.[1]

TYPES OF DIABETES
DIABETES INSIPUDUS
A high hypotonic urine output of more than 50 ml per kg of body weight per 24 hours, along with accompanying polydipsia of more than 3 liters per day, characterizes diabetes insipudus (DI), a condition.[2]Inadequate secretion and typically insufficient arginine synthesis cause central DI. Either the hypothalamus or pituitary glands contain vasopressin (AVP). Additional underlying causes of DI can result from primary forms (of renal origin) or secondary forms of polyuria in addition to central DI (resulting from primary polydipsia). These are all variations of the Polyuria Polydipsia syndrome. (PPS).

The majority of times, central and nephrogenic DI are acquired, although congenital variants can also occur due to genetic abnormalities in the AVP gene (central DI) or the AVP V2R or AQP2 water channel genes (nephrogenic DI). Polydipsia primary (PP) as A secondary form of polyuria is characterised by an excessive fluid intake that results in polyuria even in the presence of normal AVP secretion and a proper antidiuretic renal response.[3]

Etiology
A DI can be broken down into four primary categories of flaws. The most prevalent type of diabetes is central diabetes, which results from insufficient AVP secretion and synthesis by the posterior pituitary in response to osmotic stimulation. Most often, the neurohypophysis is

destroyed as the reason of this through a variety of acquired or congenital lesions under pressure or through infiltration.

Reduced renal sensitivity to the antidiuretic impact of physiological levels of AVP results in a second type of DI. A hereditary or acquired impairment in the renal processes that control urine concentration can result in this nephrogenic version of the disease.[4]

Primary polydipsia is the clinical syndrome's third cause. It is brought on by excessive, osmotically unrelated fluid consumption, which physiologically suppresses AVP secretion. To distinguish it from the other varieties of DI, such as secondary polydipsia, it is known as primary polydipsia. Happens as a result of water loss. There is a tiny minority of patients with Primary polydipsia known as having dipsogenic DI, who present with the condition in addition to psychiatric patients and health enthusiasts and whose polydipsia appears to be caused by an abnormally low thirst threshold.

The fourth kind is gestational DI, which has a similar appearance to central nervous system DI because of enhanced AVP breakdown by the placenta enzyme vasopressinase.[5,6]

Patients may occasionally have a tendency to DI due to an underlying, asymptomatic AVP deficit.[7,8]

Signs & symptoms

Diabetes insipidus is characterised by a frequent need to urinate, even during the night.

- Frequent passing of significant volumes of light-colored or clear urine.
- Constantly feeling thirsty and consuming beverages.

People with diabetes insipidus can produce up to 20 quarts of urine daily, compared to the average person's one to three quarts daily.

Dehydration can occur very fast if diabetes insipidus is not treated or if a person with the condition stops consuming fluids.

Dehydration can cause the following symptoms.

- Dizziness or lightheadedness.
- Exhaustion.
- Experiencing dry lips, eyes, and mouth.
- Challenges with simple mental tasks.
- Vomiting.
- Stagnation.[9]

Risk factors

Middle DI. Both traumatic and non-traumatic causes of harm to AVP-producing magnocellular neurons in the hypothalamus are risk factors for central DI. Surgical removal of tumours in the sellar and peritoneum is the most frequent risk factor for trauma-induced central DI. Region suprasellar.[9]

Genetic mutations, granulomatous disorders that infect the hypothalamus, such as sarcoidosis and Langerhans and non-Lagerhans cell histiocytosis, and primary brain tumours that invade or compress the hypothalamus are risk factors for non-traumatic central DI. Secondary tumours in the pituitary gland or pituitary stalk (typically metastatic breast or lung tumours), secondary tumours in the hypothalamus (such as germinoma, meningioma, craniopharyngioma, and lymphoma), and lymphocytic infundibuloneurohypophysitis (LIN), which results in the autoimmune destruction of AVP neurons.[10]

Kidney-related DI The most frequent risk factors for nephrogenic DI, aside from genetic risk factors, are lithium therapy, hypercalcaemia, and hypokalaemia.[11]

First-degree polydipsia Rarely are primary polydipsia patients with psychotic illnesses Instead of admitting they are thirsty, they offer irrational justifications for their excessive drinking or claim that alcohol makes them feel better or less anxious.[12]

Geographical DI. The peak of placental vasopressinase synthesis, which occurs at the end of the second or beginning of the third trimester, is when gestational DI frequently occurs36. Due to the relationship between vasopressin as production and placenta size, twin and multiple pregnancies have an increased risk of gestational DI.[13]

DIABETES MELLITUS

Diabetes mellitus (DM), commonly referred to as just diabetes, is a collection of conditions marked by persistently high blood sugar levels. Diabetes results from either insufficient insulin production by the pancreas or improper insulin utilization by the body's cells. The three primary kinds of diabetes mellitus are as follows.

Type 1 DM: body's inability to create enough insulin causes type 1 diabetes. Previously, this condition was referred to as "juvenile diabetes" or "insulin-dependent diabetes mellitus" (IDDM). There is no known cause.

Type 2 DM: resistance, a disease in which cells do not respond to insulin as they should, is the first sign of type 2 diabetes. In addition, a shortage of insulin may occur as the condition worsens. NIDDM (non-insulin-dependent diabetes mellitus) or "adult-onset diabetes" was the prior name for this type.

Gestational diabetes: third major kind of diabetes, known as gestational diabetes, affects pregnant women who have never had diabetes and who experience high blood sugar levels.[14]

Etiology

Diabetic causes include.
Obesity
Glucocorticoids in excess
More growth hormone
Disorders of polycystic ovaries

Change in insulin
Lipodystrophy

Type -1 DM causes

1. A genetic propensity to develop type 1 diabetes is one of the causes of the disease.
2. Particular viruses (e.g. German measles or mumps)
3. External elements

Type 2 DM causes

1. When the pancreas stops producing enough insulin or when the body becomes resistant to insulin, type 2 diabetes can occur.
2. Blood sugar amounts
3. Diabetes's time course.[15]

Signs & symptoms

Weight loss, frequent urination, increased thirst, and polyphagia is the typical signs of untreated diabetes (increased hunger).

In type 1 diabetes, symptoms might appear suddenly (weeks or months), whereas typically Type 2 diabetes develops significantly more slowly and may be undetectable or absent. Although they are not specific to the disease, a number of additional signs and symptoms can indicate the beginning of diabetes.

They also include fatigue, headaches, blurred vision, itchy skin, and poor wound healing in addition to the previously mentioned symptoms. Long-term high blood sugar levels can cause the lens of the eye to absorb glucose, changing its shape and impairing vision. There are numerous skin rashes that can happen and are collectively referred to as diabetic dermadromes.[14]

THE STRUCTURE AND FUNCTION OF INSULIN

Insulin has 51 amino acids and 6000 Da molecular weight in almost all species, including humans[16] The human insulin molecule consists of two polypeptide chains, one A chain and one B chain containing 21 and 30 amino acid residues, respectively.[17] These two chains are interconnected by SS (CysA7-CysB7 and CysA20-CysA19) with a disulfide bond, and an additional disulfide bond connects CysA6 and CysA11 in chain A (Figure 1). The amino acids of the two chains also participate in many non-covalent interactions.[16]

The function of insulin Insulin provides glucose homeostasis by keeping the plasma glucose value in an optimal range throughout the day. The main effects of insulin are: (i) In the liver, to stimulate glucose oxidation and storage of glucose (glycogenesis), as well as to convert glucose into triglycerides and protein synthesis, (ii) in the muscle tissue, it provides glucose uptake into the cells, and be stored as glycogen, (iii) and in fat tissue, it provides glucose uptake and conversion to triglycerides for storage.[18,19]

Pathophysiology

DM is characterized by complex pathogenesis and varied presentation and any classification of this disorder, therefore, is arbitrary, but nevertheless useful, and is often influenced by the physiological conditions present at the time of assessment and diagnosis. The classification currently used is based on both the etiology and the pathogenesis of the disease and is useful in the clinical assessment of the disease and for deciding the required therapy.

Type 1 diabetes mellitus

The pathogenesis of this autoimmunity, though not yet fully understood, has been found to be influenced by both genetic and environmental factors. The rate of development of this pancreatic β-cell-specific autoimmunity and the disorder itself is rapid in most of the cases as in infants and children (juvenile onset) or may be gradual as in adults (late onset).

The variability in the rate at which the immune-mediated destruction of the pancreatic β-cells occurs often defines the eventual progression of this disease. In some cases, in children and adolescents, the β-cell destruction and subsequent failure occur suddenly, which can lead to diabetic ketoacidosis (DKA), often described as the first manifestation of the disease. In others, the disease progression is very slow with a mild increase in fasting blood glucose levels, which assumes a severe hyperglycemic form with or without ketoacidosis, only in the presence of physiological stress conditions such as severe infections or the onset of other disorders. In some other cases, which include adults, β-cells may retain some degree of function to secrete only that quantity of insulin, which is only sufficient to prevent ketoacidosis for many years. However, due to progressive insulin deficiency, these individuals become insulin-dependent with the emergence of severe hyperglycemia and subsequent ketoacidosis. Despite the variable progression of this type of diabetes, the affected individuals in the beginning or in the middle, or even in the later stages of their life become severely or absolutely insulin-deficient and become dependent on insulin treatment for their survival. This severe or absolute insulin deficiency irrespective of its occurrence at any age manifests itself as low or undetectable levels of plasma C-peptide.[20,21,22]

T1DM is an autoimmune disorder characterized by several immune markers, in particular autoantibodies. These autoantibodies are associated with immune-mediated β-cell destruction, characteristic of this disease. The autoantibodies include glutamic acid decarboxylase autoantibodies (GADAs) such as GAD65, islet cell autoantibodies (ICAs) to β-cell cytoplasmic proteins such as autoantibodies to islet cell antigen 512 (ICA512), autoantibodies to the tyrosine phosphatases, IA-2 and IA-2α, insulin autoantibodies (IAAs), and autoantibodies to islet-specific zinc transporter isoform 8 (ZnT8). At least one of these autoantibodies can be used for the

clinical diagnosis of this disease but usually, more of these immune markers have been observed in approximately 85–90% of patients with new-onset T1DM.[20,23] Of these autoantibodies, GAD65 is the most important and is present in about 80% of all T1DM individuals at the time of diagnosis, followed by ICAs present in 69–90% and IA-2α found in 54–75% of all T1DM individuals at clinical presentation.

The IAAs are important immune markers present in infants and young children who are prone to diabetes and their prevalence decreases as the age of onset of diabetes increases. The presence of IAAs in these individuals who have not been previously treated with insulin is an important indication of developing T1DM. IAAs are present in about 70% of all infants and young children at the time of diagnosis. The IAAs also plays an important inhibitory role toward insulin function in patients on insulin therapy. Although not often clinically significant but nevertheless, this immune response has been observed with varying degrees of severity in at least 40% of patients on insulin treatment and therefore shows differential clinical manifestations.[24] These autoantibodies mostly consist of polyclonal immunoglobulin G (IgG) antibodies and differ in their affinities and binding capacities toward insulin. IAAs can either be high insulin affinity/low insulin-binding capacity or low insulin affinity/high insulin-binding capacity. The low insulin affinity/high insulin-binding capacity IAAs are responsible for clinical manifestations. At high titers, the binding of these antibodies to insulin prevents or delays its action and is responsible for characteristic hyperglycemia in the immediate postprandial period, which leads to significantly increased insulin requirements followed by unpredictable hypoglycemic episodes (postprandial hypoglycemia) observed later.[25]

Idiopathic diabetes

Idiopathic diabetes, also referred to as ICA-negative or type 1B diabetes, includes the forms of diabetes which are similar to T1DM in presentation but characterized by variable nonimmune β-cell dysfunction without any observed HLA association unlike T1DM and hence, sometimes it is also described as a separate type of DM. This type of diabetes exhibits a strong pattern of inheritance and has been observed in only a minority of patients, of Asian or African-Caribbean origin. The etiology of idiopathic diabetes remains largely unknown.

The disease is characterized by severe but varying degrees of insulin deficiency (insulinopenia) which can exhibit episodic patterns concomitant with varying degrees of severity and episodic DKA. These patients, therefore, may require insulin replacement therapy initially but the need for the therapy may not be absolute and may vary in accordance with the episodic patterns of insulinopenia and ketoacidosis characteristic of these forms of T1DM.[26]

Type 2 diabetes mellitus

T2DM, also known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes, as per the previous nomenclature, constitutes about 90–95% of all cases of diabetes. This type of diabetes is characterized by two main insulin-related anomalies: insulin resistance and β-cell dysfunction.[27,28,29] Insulin resistance results from the disruption of various cellular pathways, which leads to a decreased response, or sensitivity of cells in the peripheral tissues, in particular the muscle, liver, and adipose tissue toward insulin. In the early stages of the disease, decreased insulin sensitivity triggers β-cells hyperfunction to achieve a compensatory increase in insulin secretion to maintain normoglycemia. The higher levels of circulating insulin (hyperinsulinemia), thus, prevent hyperglycemia. However, gradually, the increased insulin secretion by β-cells is not able to compensate sufficiently for the decrease in insulin sensitivity. Moreover, β-cell function begins to decline and β-cell dysfunction eventually leads to insulin deficiency. As a result, normoglycemia can no longer be maintained and hyperglycemia develops. Although insulin levels are decreased, the secretion of insulin in most cases is sufficient to prevent the occurrence of DKA.[29] But DKA may occur during severe stress conditions such as those associated with infections or other pathophysiological scenarios. DKA may also be precipitated by the use of certain drugs including sodium-glucose co-transporter-2 (SGLT2) inhibitors, corticosteroids, and atypical antipsychotics (second-generation antipsychotic drugs).[30,31] In absence of any severe physiological stress conditions, patients with T2DM often do not require any insulin therapy both at the time of disease onset and even after, throughout their lifetime.[27.28.29,]

T2DM progresses very slowly and asymptomatically with even mild hyperglycemia developing over years and as such remains largely undiagnosed until the appearance of classic symptoms associated with severe hyperglycemia such as weight loss, growth impairment, blurred vision, polyuria, and polydipsia in the advanced stages of the disease. The pathogenesis/etiology of this form of diabetes is complex and involves multiple known and unknown factors, which in a conclusive manner can be described as a combination of genetic (polygenic) predispositions and strong environmental influences. T2DM has been more frequently associated with increasing age, obesity, family history of diabetes, physical inactivity, and adoption of modern lifestyles: with prior GDM in women and with pathophysiological conditions such as hypertension and dyslipidemia. It occurs more frequently in individuals belonging to certain racial or ethnic groups including Native Americans (American Indians), Asian Americans, African Americans, Hispanics, and Latino. The frequent occurrence of T2DM in the mentioned racial or ethnic groups and its observed strong association with first-degree blood relations point strongly toward the role of genetic factors in the etiology of this disease, but these

factors are complex and remain largely unspecified. However, unlike T1DM, no association of this disease has been found with genes involved in the immune response including autoimmunity and consequently there is no immune-mediated pancreatic β-cell destruction.[30,31]

Obesity plays an important role in the homeostatic regulation of systemic glucose due to its influence on the development of insulin resistance through its effect on the sensitivity of tissues to insulin and as such most but not all patients with T2DM are overweight or obese.[32] The increased body fat content, a characteristic of obesity, is such an important risk factor for T2DM that not only the total amount but also the distribution of body fat itself defines the development of insulin resistance and subsequently hyperglycemia. Increased abdominal fat or visceral obesity has been frequently associated with this type of diabetes in comparison to increased gluteal/subcutaneous fat or peripheral obesity.[35] Due to its strong association with increased body fat content or obesity, patients with T2DM often present with various cardiovascular risks factors such as hypertension and lipoprotein metabolic abnormalities characterized by elevated triglycerides and low levels of high-density lipoproteins (HDLs). Due to its lifelong duration and associated diverse metabolic derangements characteristic of hyperglycemia, T2DM, particularly in the middle and later decades, is frequently associated with the development of various microvascular and macrovascular complications.

PHARMACOLOGICAL SCREENING OF ANTIDIABETIC DRUGS IN VIVO MODELS

I. Models for Insulin Dependent Diabetes Mellitus [IDDM]

1. Alloxan-induced diabetes

Principle

The cyclic urea molecule alloxan causes lifelong diabetes. It is a highly reactive chemical that damages beta islet cells with free radicals and kills them. Alloxan exhibits extraordinary beta-cell selectivity when exposed to islets in vitro, with the other islet cells essentially escaping both its inhibitory and lethal effects. Alloxan has been found to change the characteristics of the beta cell plasma membrane in numerous investigations.

High glucose concentrations counteract the effects of both the aberrant membrane shape and changed ion flux in mouse islets treated in vitro with alloxan. Although alloxan affects the plasma membrane, other medications' effects on the cellular and molecular elements of beta cells may also cause these alterations. Alloxan interacts with sulfhydryl-containing cellular elements after being taken up by beta cells, especially sulfhydryl enzymes that are known to be crucial for beta-cell function. Alloxan is particularly sensitive to inhibiting the enzyme glucokinase, which has a signal-recognition role in connecting glucose levels to insulin secretion. According to research, alloxan's primary intracellular target and ultimate cause of cytotoxicity may be the sulpha hydryl groups of glucokinase. In greater quantities, alloxan also inhibits enzymes like protein kinase and hexokinase. The direct induction of mitochondrial abnormalities, the extreme sensitivity of beta cells to the cytotoxic effects of free radicals (produced during the reduction/re-oxidation cycle of alloxan), and DNA damage within the beta cell nucleus are other proposed mechanisms for how alloxan can cause cytotoxicity. Alloxan causes DNA to fragment both in vivo and in vitro. This drives nuclear poly (ADP-ribose) synthase to repair the DNA, which depletes cellular NAD and impairs beta cell activity. In conclusion, alloxan may have an impact on the beta cell's plasma membrane, mitochondria, and nucleus, among other places.

➢ **Procedure**

A single injection of alloxan monohydrate, 100 mg/kg body weight, diluted in normal saline, is given to albino rats of either sex, weighing 150–200g.

• Animals are held for 48 hours, during which time they are given unlimited access to food and water.

• Blood glucose levels exhibit a triphasic reaction, with hyperglycaemia lasting an hour, followed by six hours of hypoglycaemia, and then 48 hours of sustained hyperglycaemia.

• Diabetic animals are those in which, 48 hours after receiving alloxan, fasting blood glucose levels are above 140 mg/dl.

Drug samples for screening are given orally for a six-week period.

Animals are fasted for eight hours before blood samples are taken through the caudal vein after six weeks of treatment.

• For ten minutes, the serum is centrifuged (3000 rpm) while being cooled (2-4 °C).

• Using an autoanalyzer and the glucose oxidase-peroxidase method [GOD-POD kit], the serum glucose level is calculated.[36]

2. Streptozotocin induced diabetes

Principle:

Theoretically, the broad-spectrum antibiotic streptozotocin damages beta islet cells by generating free radicals. With the exception of rabbits and guinea pigs, it causes diabetes in practically all animal species. Streptozotocin can cause diabetes when it is administered as a single large injection (like alloxan) or as several smaller doses.

Single Dose Streptozotocin Diabetes

Streptozotocin may harm the beta cell membrane, causing alterations comparable to those generated by alloxan, and may have several similar beta cytotoxic pathways with this drug. Streptozotocin is also believed to function intracellularly, where it may reduce the amount of NAD present in islets.

Alloxan and streptozotocin both have the capacity to cause DNA strand breaks in beta cells. A cascade of

intracellular activities, including the stimulation of DNA repair (through poly-ADP-ribose synthase), a decrease in the NAD content of the islets, and subsequent suppression of the islet functions, are also triggered by the streptozotocin-induced lesions. Despite some evidence to the contrary, streptozotocin and alloxan may not share the same mechanism through which they cause beta cytotoxicity.

Multiple Low-Dose Streptozotocin Diabetes

Repeated sub-diabetogenic dosages of streptozotocin injections can cause diabetes in mice, and this condition is accompanied by severe pancreatic insulitis, suggesting that cell-mediated immunity may be involved in the pathogenesis and suggesting similarities to human IDDM.

Studying the manner in which the immune system may enhance the impact of beta cytotoxic agents but not the spontaneous development of IDDM is possible using streptozotocin-induced diabetes.

➢ **Procedure**

Streptozotocin is produced in citrated buffer with a pH of 4.5 (60 mg/kg body weight).

• The aforementioned fluid is intraperitoneally administered into 150–200 g albino rats of either sex.

• Diabetic animals are those with fasting blood glucose levels > 140 mg/dl after receiving streptozotocin for 48 hours.

• After six weeks of therapy, caudal vein blood samples are taken from animals who have been fasted for six hours.

• For ten minutes, the serum is centrifuged (3000 rpm) while being cooled (2-4 °C).

• Using an autoanalyzer and the glucose-peroxidase method [GOD-POD kit], the serum glucose level is calculated.[37]

3. Virus-induced diabetes

One of the etiological agents for IDDM is viruses. They infect and kill the pancreatic beta cells, resulting in diabetes mellitus. The human viruses RNA picornavirus, encephalomyocarditis (EMC-D), and coxsackie B4 (CB-4) are among those utilised to cause diabetes.

➢ **Procedure**

0.1 ml of D-variant encephalomyocarditis [EMC] at a 1:50 dilution is injected intraperitoneally (i.p.) into 6–8-week-old mice.

50 PFU (plaque-forming units) of the EMC virus are present in 0.1 ml of the aforementioned dilution. (This viral concentration causes about 10-20% of deaths.)

A less infectious variation causes comparable harm by inducing an autoimmune response against the beta cells.

If the non-fasting levels of infected animals are 250 mg/dl or higher than the levels of uninfected animals of the same strain, the animals are deemed to be hyperglycemic.

Drug screening samples are given orally for a total of 6 weeks.

Drug screening samples are given orally for a total of 6 weeks.

In order to assess the anti-diabetic effects of the drugs, a blood glucose estimate is performed after six weeks of treatment.[37,38]

4. Hormone-induced Diabetes Mellitus

A long-acting glucocorticoid, dexamethasone is an immunosuppressive steroid that promotes an autoimmune reaction in the islets and results in type 1 diabetes.

➢ **Procedure**

Dexamethasone is intraperitoneally injected twice daily into adult rats weighing 150–200 g. The dose is 2–5 mg/kg.

IDDM is caused by administering the same dose level repeatedly over a 20–30-day period.

A suitable route is used to administer the material that will be examined.

To ascertain the activity, blood glucose is tested.[39]

5. Insulin antibodies induced Diabetes Mellitus

By administering anti-insulin serum to guinea pigs, a transitory diabetes state can be produced.

Diabetes continues as long as antibodies are able to react with any insulin that is still in circulation.

Antibody preparation Guinea pigs weighing 300 to 400 g are injected with 1 mg of bovine insulin that has been dissolved in acidified water (pH 3.0).

After two weeks of antigenic exposure, anti-insulin sera are obtained.

➢ **Procedure**

Adult albino rats are administered an intravenous injection of 0.25–1.0 ml of guinea pig anti–insulin serum.

A dose-dependent rise in blood glucose levels of up to 300 mg/dl is brought on by insulin antibodies.

The drug sample being tested is given by a suitable method, and the activity is determined by analysing the blood glucose level.

Large doses and extended administration, however, cause the animals to die by causing acidosis, glycosuria, ketonuria, and ketonemia. After receiving lesser doses, the diabetic state can be reversed.[40]

6. Genetic model

1. The Non-Obese diabetic model (NOD model)

Developed by Makino and colleagues in Japan, Non-obese Diabetic (NOD) mice are an inbred type of albino mice.

➢ **Procedure**

It is developed from breeding JCL: NOD mice and ICR (Swiss mouse) progenitors are the results of more than 80 generations of sib mating.

The strain was kept in the first 20 generations of sib mating as a control for normoglycemia to match with a

different line being chosen for impaired glucose tolerance (NONstrain).
The development of frank hyperglycaemia and glycosuria, rather than non-glycemia, became the chosen phenotype after the spontaneous development of lDDM was discovered in a female of the control NOD strain at F20.[41]

2. Bio-breeding rat.
Diabetes that develops without warning in the BB The Chapel brothers made the first Wistar rat diagnosis in 1974 at the Bio breeding Laboratories commercial breeding facility in Ottawa, Ontario, Canada in a non-inbred but closed outbred colony of Wistar rats.
There is a hereditary susceptibility to the condition, as well as a protracted pre-diabetic phase followed by a rapid start of symptoms at three months of age.
After the initials of the breeding lab, it was chosen to call this syndrome BB.

➢ **Procedure**
Over the course of 20 generations, rats are bred in the laboratory using sib mating.
IDDM develops spontaneously in rats after 20 generations of sib mating.
The clinical manifestation of diabetes in the BB rat is comparable to that in humans. Within a day of the commencement, substantial hyperglycemia, glycosuria, and weight loss take place and are linked to decreasing plasma insulin levels, which, if ignored, will cause ketoacidosis within a few days.
The BB rat is one of the rare rodent models, like the NOD mouse, in which considerable ketosis happens even in the absence of obesity.[42]

II. MODELS FOR NIDDM
a. Chemically Induced Diabetes
1. Neonatal STZ Model of NIDD
The significant death of pancreatic beta cells by streptozotocin is accompanied by a fall in pancreatic insulin reserves and an increase in plasma insulin levels.

➢ **Procedure**
STZ (80 to 100 mg/kg i.p.) is administered to new born Wistar or Sprague-Dawley strain rats at birth or within the first five days after birth.

Increased plasma glucose levels, decreased pancreatic insulin reserves, and significant pancreatic beta-cell death are all present.

However, the B-cells of the neonates treated with STZ partially regenerate in contrast to adult rats treated with STZ.
The STZ-heated neonatal rat becomes normoglycemic at 3 weeks of age after an initial plasma glucose increase.
The proliferation of cells produced from ducts is the primary cause of the B-cell number increase in the following few weeks; the magnitude of this increase depends on the age at which the animal receives STZ treatment as well as the type of treated rat.[43,44]

2. Dithizone-induced Diabetes Mellitus
In mice, cats, rabbits, golden hamsters, and rabbits, dithizone produced the signs and symptoms of diabetes. Serum levels of zinc, iron, and potassium were found to be above average in dithizonized diabetic mice, although copper and magnesium levels remained unaltered. Except for blood potassium and magnesium, most of these serum values returned to normal following insulin therapy.[45]

➢ **Procedure**
In the islets of Langerhans, organic compounds (8-(p-toluenesulfonylamino) quinoline) (TSQ) react with zinc, causing the death of islet cells and diabetes.
A triphasic glycaemic response to dithizone injection will occur at dose levels of 50–200 mg/kg.
Once again, hyperglycaemia is shown after 24-72 hours and lasts for a considerable amount of time.
Preliminary hyperglycaemia will be noticed after 2 hours and normoglycemia after 8 hours, which continued up to 24 hours.
The drug sample to be analyzed is administered through a suitable route and blood glucose is determined.[46]

b. Genetically induced diabetic animal model
Spontaneous diabetic animals of type-2 diabetes may be obtained from animals with one or several genetic mutations transmitted from generation to generation. These animals typically inherit diabetes as single-gene or multiple-gene abnormalities, as in the case of KK, db/db, or Zucker fatty mice and rats. The metabolic characteristics are caused by a single gene defect (monogenic), which can be recessive or dominant (e.g., diabetes or db/db mice, Zucker fatty rat) or have a polygenic origin (e.g., Kuo Kondo (KK) mouse, New Zealand obese mouse).[47]

1. Zucker Diabetic Fatty Rat
These were produced via the inbreeding of a substrain of hyperglycaemic fa/fa (leptin receptor-deficient) rats. Normal islet architecture is disrupted, ß-cell degranulation is enhanced, and ß-cell death is elevated in the Zucker diabetic fatty rat. In this strain, between 7 and 10 weeks of age, when the animals' average plasma glucose level has risen above 22 mm, all animals develop obesity, insulin resistance, and overt NIDDM. Another study has shown that male Zucker diabetic fatty rats develop type 2 diabetes on their own, whereas females develop diabetes when fed a high-fat, diabetogenic diet (HF-fZDF).[48]

2. Goto-Kakizaki rat
The Goto-Kakizaki (GK) rat, which is commonly used to investigate type-2 diabetes, provides a genetic model of the disease and exhibits significantly impaired insulin production that results in baseline hyperglycaemia. In Japan in 1973, Goto and his co-workers developed the

GK rat, a polygenic model of type-2 diabetes, through selective inbreeding of Wistar rats with impaired glucose tolerance.
Non-obesity, moderate but persistent fasting hyperglycaemia, hyperinsulinemia, normolipidaemia, decreased glucose tolerance that manifests at 2 weeks of age in all animals, and an early onset of diabetes sequelae are its defining characteristics.

But it is still unclear what the pancreatic islets of Langerhans in GK rats look like morphologically. In animals older than seven weeks, structural islet alterations were not seen. GK rats that were 14 and 21 weeks old, however, showed histological islet alterations. Islet shape generally changed, and -cell responses to anti-insulin appeared to be diffusely decreased. According to electron microscopy, there were fewer so-called -granules and more immature granules than before. A hyperfunction of the cells is shown by the Golgi apparatus of -cells and the dilated rough endoplasmic reticulum cisternae. According to this study, more complex cellular processes than simple-cell malfunction and/or degeneration are what lead to insulin insufficiency in GK rats. One of the most well-known animal models for examining the relationship between changes in beta cell mass and the incidence of type 2 diabetes and diabetic consequences is the GK rat (particularly diabetic nephropathy). However, the literature only has a very small number of studies on drug testing that used this paradigm.[49]

IN VITRO MODELS

Glucose Uptake by the Isolated Diaphragm from Mice and Rats

The effects of insulin and insulin-like compounds on muscle tissue can be studied using this paradigm.

Glycogen Synthesis Research

Rats weighing 70–100 g that are male Sprague Dawley's are used to harvest their diaphragms. The produced diaphragms are split into two equal parts. The hemidiaphragms are incubated in Krebs-Henseleit buffer, gassed with 95 percent nitrogen and 5 percent carbon monoxide, and with 5 mM (UC) glucose (0.5 mc/ml), insulin, or the substance under investigation. The hemidiaphragms are blotted on tissue and put in liquid nitrogen after being frozen for 30 minutes. Before adding ethanol at a concentration of 70%, the powdered tissue is first dissolved by boiling for 45 min at 100°C in 30% KOH (1 ml/100 mg tissue). The samples are centrifuged at 2000 g for 10 minutes after being frozen at -20 °C. Before liquid scintillation counting is used to calculate the amount of "C labelled glycogen, the glycerin pellets are rinsed three times with 70% ethanol. After hydrolysis into glucose (1 N HCL at 100°C for 3 hours), total glycogen is calculated.[50]

To Study Glucose Transport

Freshly dissected rat diaphragms are incubated for 30 min at 37 °C in HEPES-buffered salme (25 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.5 mM CaCl, 1 mM MgCl, 5 mM glucose, 0.5 mM sodium pyruvate, 1.5 mM KH.PO, pH 7.4) under constant bubbling with 95% 0/5% CO The diaphragms are then washed two times with the same buffer without glucose, and incubated further for 30 min in 5 ml of the above buffer with test compound or insulin. Glucose transport is initiated by addition of 50 ml of 10 mM 2-(1-H) deoxyglucose (10 mci/ ml) in the absence or presence of 25 mM cytochalasin B (control) .After 15 minutes, the diaphragms are homogenised, blotted with filter paper, and four times washed in an ice-cold buffer containing 10 mM glucose and 25 mM cytochalasin B. Protein determination uses a portion of the homogenate. Supernatant samples in a volume of 1 ml are centrifuged at 10,000 g for 15 minutes before being combined with a scintillation cocktail and radioactivity measured. The difference between diaphragm associated radioactivity measured in the absence (total uptake) and presence of cytochalasin is used to quantify glucose transfer (dpm/mg of protein).[51]

Glycogen Synthase Activity Research

Male Wistar rat intact hemidiaphragms are incubated in DMEM (10 ml/hemidiaphragm) at 37°C with test chemicals or insulin and 5 mM glucose while being continuously bubbled with O, CO, and N2 (95:5). The diaphragms are blotted and then frozen in liquid nitrogen to create the homogenate. In 10 vol of 25 mM tris/HCl (pH 7.4), 100 mM NaF, 5 mM EDTA, and 0.1 mM PMSF, frozen diaphragms are first pulverised in a porcelain mortar before being homogenised at 0°C. Centrifuging is done on the homogenate (10000 g, 20 min).[39]

Glycogen synthase aassay

Procedure

In 30 ml of 25 mM Tris/HCl (pH 7.4), 50 mM NaF, 10 mM EDTA (preincubated at 30 °C), and either 1 or 10 mM glucose-6-phosphate, 10 microliters of diaphragm homogenate is added. 22 mM (UC) UDP-glucose (10 mci) is added to start the reaction, and 2.5 ml of e-cold 66% ethanol is added to stop it after 15 minutes, followed by filtering on pre-wetted Whatman GF/C glass fibre discs. The hers are cleaned, dried, and their radioactivity is measured. The homogenate is added to tubes containing the entire reaction mixture together with ice-cold ethanol to assay blanks. At the stage of homogenization, the actional velocity serves as a metric for the amount of glycogen synthase that is active in vivo (1-form) ward the total enzyme contents (1-+ d-forms) a statistic was constructed comparing the activities discovered at 0.1 (1-form) and 10 mM glucose-6-phosphate (1++ 4-forms).[40]

Isolated Target Tissue

Rats with Perfused Hind Limbs
Wistar female rats weighing 170–230 g are employed. A 48-hour fast is observed before the experiment. are put to sleep by administering 50 mg/kg of pentobarbital intraperitoneally. After the aorta has been identified, a

ligature is wrapped around it above the renal vessel's origin, and it is then fastened. The left renal and iliolumbar vessels are cut into the aorta, and a No. 18 polyethylene catheter is then inserted. It is then advanced up to a point halfway between the iliolumbar vessels and the aortic bifurcation, flushed with heparin-NaCl solution, and knotted in place. A No. 16 needle is used to cannulate the vena cava, and it is fixed in place. so that the portic catheter's tip is at the same level as its tip. Vinyl tubing is attached to the needle. After that, the prepared is moved to a perfusion device. The tubing carrying the oxygenated medium has an aortic cannula attached to it. Krebs-Ringer bicarbonate buffer, 45 g of bovine serum albumin, and 10 mmol/1 D-glucose make up the perfusion medium. 70 ml are utilised for recirculation during a two-hour period, and it is perfused at a rate of 8 ml/min. The perfusate medium is supplemented with the test chemicals at a concentration of 40–100 mmol/l. The perfusate's pH value determines the ratio of bubbled CO,/O, which is bubbled at a rate of 70 ml gas mixture/min. A detergent with a low foaming rate (14 ml/ml 0.1% Genapol PF-10) to the perfusate is added.[52]

Muscle Cell Lines

BC3H1 Myocytes

A mouse neoplasm gave rise to the BC3H1 cell line, a nonfusing, spontaneously developing, and reversibly differentiating mouse muscle cell line. Although BC3H1 myocytes show characteristics of both smooth and skeletal muscle cells under the electron microscope, their action potential and nicotinic acetylcholine receptors are more similar to those of skeletal muscle cells.

In 100 mm dishes, confluent BC3H1 myocytes are grown for 10–14 days in Dulbecco's Modified Eagle's Media (DMEM) with 15% Process Serum Replacement-1 and 25 mM glucose (added 18 h before the experiment). Rinsed and pre-incubated for 20 minutes at 37°C in Dulbecco's phosphate buffered saline with 0.1 mM CaCl and 1 mg/ml BSA, the cells are then treated for 30 minutes with the buffer (as a control) or with agonists in the buffer. On this cell line, test substance can be investigated for: its direct impact on the transfer of glucose Skeletal muscle membrane 5'-nucleotidase activity is decreased by insulin. glucose transport connected to PKC activation similar to that of diacylglycerol.[53]

Isolated Rat Liver

Procedure

Male Wistar rats weighing 200 to 250 g are sedated intraperitoneally with 150 mg/kg hexobarbital before having their livers extracted and physiological saline solution flushed through the portal vein for three minutes at 37°C. The prepared substance is then moved to a perfusion device, where a portal vein cannula is connected to a tube carrying an oxygenated medium. Krebs-Ringer bicarbonate buffer is perfused at a rate of 30 ml/min, along with 25% cow erythrocytes, 1.6% bovine serum albumin, and 22.5 mmol/L Na-l-lactate. Throughout the course of two hours, 70 ml are consumed for recirculation. The perfusate medium receives an addition of the test substance at a concentration of 40–100 mol/l. For gassing, a variable CO/O mixture is utilised, the ratio of which depends on the perfusate's pH level. A 70 cc gas combination is bubbled into the perfusate per minute. The perfusate needs to have a detergent added to prevent foam formation. The catheter is used to remove the samples for analysis."[54]

Isolated Hepatocytes

Procedure

Hepatic cell suspension at a concentration of 10 cells/ml is perfused with Hanks 10 mM HEPES buffer solution (pH 7.5, 0.5% BSA, and 1 mM palmityl oleate) for 10 minutes at 37°C. The supernatant is removed after the cells have been centrifuged at 40 g for 15 sec. The remaining combinations are then combined with the same buffer and varied quantities of the test substance, and they are then incubated for 10 minutes at 37°C. Cooling on ice stops the reaction. The mixture is centrifuged for 60 seconds at 170 g at 4 °C. A buffer solution containing 1 mM EGTA and 10 mM MgCl is used to homogenise the pellet. The homogenate is combined with an equal volume of 400 mM Tris-HCl buffer following 20 minutes of heating at 80°C. (pH 7.5) then centrifuged at 17,500 g for 5 minutes at ambient temperature. Fructose-26- biphosphate is measured in the supernatant by mixing it with a solution containing 54 mM Tris-HCl, 1 mM Fructose-6 phosphate, 0.2 mM NADH, 7.5 mM dithiothreitol, 0.5 mM EDTA, 0.01 U phosphofructokinase, 0.4 U aldolase, 1 U glycerine-3- By using colorimetry, the amount of fructose-2,6-biphosphate in the sample solution is quantified, and the reaction rate of a known amount of fructose-2,6-biphosphate is compared.[55]

CONCLUSION

In conclusion, diabetes is a condition marked by a partial or complete lack of insulin, which results in hyperglycemia. Type 1 diabetes and type 2 diabetes are the two basic kinds of disease. In contrast to type 2 diabetes, which is brought on by insulin resistance and a failure of the beta cell to adjust, type 1 diabetes results from an autoimmune attack on the insulin-producing pancreatic beta cells. Animals that naturally develop autoimmune diabetes to those whose pancreatic beta cells have been chemically removed are used as animal models for type 1 diabetes. With variable degrees of insulin resistance and beta cell failure, type 2 diabetes is modeled in obese and non-obese animal models. Some of the models currently employed in diabetes research are described in this review. Ideally, multiple animal models should be utilized to accurately depict the variety of human diabetic patients.

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