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BIOLOGICAL SCREENING PROCEDURES FOR ANTI-DIABETIC DRUGS-A REVIEW

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ABSTRACT

Appropriate experimental models are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of type 2 diabetes and testing of various therapeutic agents. The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and/or by combination thereof. This review gives an overview on the animal models of type 2 diabetes with reference to their source, characteristic features, underlying principal and procedure to the investigators in diabetes research.

KEYWORDS: Diabetes is a complex and heterogeneous disorder presently.

INTRODUCTION

Diabetes is a complex and heterogeneous disorder presently affecting more than 100 million people worldwide and causing serious socio-economic problems.

Appropriate experimental models are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of type 2 diabetes and testing of various therapeutic agents. The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and/or by combination thereof. This review gives an overview on the animal models of type 2 diabetes with reference to their source, characteristic features, underlying principal and procedure to the investigators in diabetes research.[1,2]

Diabetes can be divided into two groups based on their requirements for insulin: -

Type 1: Insulin- dependent diabetes mellitus [IDDM]

Type 2: Non- insulin dependent diabetes [NIDDM]

Type 1: Insulin dependent diabetes mellitus [IDDM]

Cause: A burst of insulin secretion normally occurs after ingestion of a meal in response to transient increase in the levels of circulating glucose and amino acids. in the post operative period, low, basal levels of circulating insulin are maintained through beta cell secretion. However type one diabetic has virtually no functional beta cells.

Treatment: Type 1 diabetic must rely on exogenous (injected) insulin in order to control hyperglycemia, maintain acceptable levels of glycosylated hemoglobin (HbA_1C) and avoid ketoacidosis. The goal in administering insulin to type 1 diabetic is to maintain blood glucose concentrations as close to normal as possible and to avoid wide swings in blood glucose levels that may contribute to long-term complications.

Type 2: Non- insulin dependent diabetes mellitus [NIDDM]

Most diabetics are in this category, metabolic alterations observed are milder than those described for IDDM [e.g. NIDDM patients typically are not ketotic], though long-term clinical consequences can be just as devastating e.g. vascular complications and subsequent infection can lead to amputation of the lower limbs.

Cause: In NIDDM pancreas retains some beta cell function, resulting in variable insulin levels that are insufficient to maintain glucose homeostatis. Patients with type 2 diabetes are often obese.

Type 2 diabetes is frequently accompanied by target organ insulin resistance that limits responsiveness to both endogenous and exogenous insulin. In some cases insulin resistance is due to a decreased number of mutations of insulin receptors.

Treatment: The goal in treating type 2 diabetes is to maintain blood glucose concentrations with normal limits and to prevent the development of long-term complications of the disease. Weight reduction, exercise and dietary modification decrease insulin resistance and correct the hyperglycemia of type 2 diabetes in some patients. Oral hypoglycemic agents & insulin therapy may be required to achieve satisfactory serum glucose levels [1,2,7].

Insulin

Insulin is a small protein consisting of two polypeptide chains that are connected by disulphide bonds. It is synthesized as precursor protein [pro insulin] that undergoes

proteolytic cleavage to form insulin and peptide C, both of which are secreted by beta cells of pancreas. Normal individuals secrete less pro insulin than insulin, whereas NIDDM patients.

Insulin secretion

Insulin secretion is regulated by blood glucose levels, hormones and autonomic mediators. Secretion is most commonly triggered by high glucose which is triggered by high blood glucose which is taken up and phosphorylated in the beta cells of the pancreas. Adenosine triphosphate [ATP] levels rise and blocks the potassium channels, leading to membrane depolarization and an influx of calcium ions which causes pulsatile insulin exocytosis.

Sources of insulin

Insulin is isolated from beef and pork pancreas. Human insulin is replacing the animal hormone for therapy. Human insulin is produced by special strain of E. coli that has been genetically altered to contain the gene for human insulin. Pork insulin is closest to human insulin, differing by only one amino acid.

Insulin administration

Since insulin is a protein it is degraded in the gastrointestinal tract if taken orally. It is generally administered by subcutaneous injection.

Adverse reactions of insulin

The symptoms of hypoglycemia are the most serious and common adverse reactions to an overdose of insulin i.e. tachycardia, confusion, vertigo, diaphoresis, lipodystrophy, hypersensitivity etc.

Secrete high levels of pro hormone, Since radioimmunoassay do not distinguish between the two insulin types, NIDDM patients may have lower levels of active hormone than the assay indicates. The measurement of circulating C peptide provides a better index of insulin levels.[1,3]

Animals Used For The Screening Of Anti-Diabetic Drug[4,6,7]

Obese mouse, Diabetic mouse,Sand mouse [Psammomys obesus], Spiny mouse [Acomys cahirinus]

BB rats, KK mouse, Yellow mouse, Yellow KK mouse

New Zealand obese mouse,Tuco-tuco [clenomys talarum]- these are burrowing rodents from Argentina. Chinese hamster [Cricetulus griseus], NOD mouse, Japanese wistar rat [Goto rat] etc.

Chemical Agents Capable Of Inducing Diabetes[5,6,7]

A) Irreversible beta cytotoxic agents:

- Alloxan
- Steptozocin
- Diphenyl thiocarbazine
- Oxine-9- hydroxyquinolone
- Vacor

B) Reversible beta cytotoxic agents

- 6- aminonicotinamide
- l-asparginase
- Azide
- Cyanide
- Cyproheptadine
- Phenyloin
- Thiazides
- Malonates

C) Other agents

- Anti-insulin antibodies
- Somatostatins
- Catecholamines
- Glucocorticoids
- Glucagoan.

1. Models For Insulin Dependent Diabetes Mellitus [IDDM][1,2,8,10,15,21]

1.1 Alloxan induced diabetes

Principle

- Alloxan: is a cyclic urea compound, which induces permanent diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells & causes cell death.

- When islets are exposed in vitro to alloxan, it exhibits exceptional beta cell specificity, the other islets cells remaining largely unaffected by both it's inhibitory and cytotoxic effects.
- Several studies have shown that alloxan alters the properties of beta cell plasma membrane.
- In rodents islets treated in vitro with alloxan displays abnormal membrane morphology & altered the ion flux, both effects being prevented by high glucose concentrations.
- Although alloxan has an effect at plasma membrane, these changes may be secondary to actions of drugs on the cellular and molecular components of the beta cells.
- Following it's uptake by the beta cells, alloxan interacts with sulphydrl- containing cellular components, particularly sulphydryl enzymes known to be essential for beta cell function.
- Glucokinase, an enzyme which has signal-recognition function in coupling the glucose concentration to insulin release is particularly sensitive to inhibition by alloxan.
- Findings have led to hypothesis that the sulphydryl groups of glucokinase may be primarily the intracellular target for alloxan and responsible ultimately for it's cytotoxicity.
- Enzymes like hexokinase, protein kinase are also inhibited by alloxan at higher concentrations.
- Other proposed mechanism for alloxan cytotoxicity include direct induction of mitochondrial abnormalities, extremesensitivity of beta cells to the cytotoxic effects of free radicals (generated during the reduction/ re-oxidation cycle of alloxan) & damage to DNA within the beta cell nucleus.
- Alloxan induces fragmentation of DNA both in-vivo & in-vitro, which stimulates DNA repair by nuclear poly (ADP-ribose) synthetase, leading to delpletion of cellular NAD and impaired beta cell function.
- IN SUMMARY, alloxan may exert its effect at several sites including the plasma membrane, mitochondria and nucleus of the beta cell.

Dose: - In rats Alloxan at dose of 100 mg/kg produces diabetes

In rabbits dose of 150 mg/kg infused through marginal ear vein produces diabetes in 70% of the animals.

Procedure: -

- Albino rats of either sex [150-200g] are injected with a single dose of alloxan monohydrate [100 mg/kg body weight] dissolved in normal saline by i.p. route.
- Animals are kept for 48 hours during which food and water is allowed ad libitum.
- Blood glucose levels show triphasic response with hyperglycemia for one hour followed by hypoglycemia that lasts for six hours & stable hyperglycemia after 48 hours.
- Animals showing fasting blood glucose level above 140 mg/dl after 48 hour of alloxan administration are considered diabetic.
- For a period of six weeks, drug samples to be screened are administered orally.
- After six weeks of treatment, blood samples are collected from 8 hour fasting animals through a caudal vein.
- Serum is separated by centrifuge (3000 rpm) under cooling (2-4 °C) for ten minutes.
- The serum glucose level is estimated by glucose oxidase-peroxidase method [GOD-POD kit] using autoanalyser.

1.2 Streptozotocin induced diabetes

Principle: -

Streptozotocin: is a broad-spectrum antibiotic, which causes beta islet cell damage by free radical generation. It induces diabetes in almost all species of animals excluding rabbits and guinea pigs.

Diabetes can be induced by Streptozotocin when it is given either as single large (as with alloxan) or as multiple sub diabetogenic injections.

Single Dose Streptozotocin Diabetes

- Streptozocin may share several common Beta cytotoxic mechanisms with alloxan.
- Streptozotocin may damage the beta cell membrane, producing changes similar to those induced by the alloxan.
- Streptozocin is also thought to act intracellularly, where it may deplete the islet content of NAD.
- Streptozotocin shares with alloxan the ability to induce strand breaks in beta cell DNA. Moreover the induction of these lesions by streptozotocin is followed by a cascade of intracellular events similar to those provoked by exposure to alloxan i.e. stimulation of DNA repair (via poly- ADP- ribose synthetase), reduction of islet NAD content and subsequent inhibition of the islet functions.

- Despite some evidence that streptozotocin and alloxan exert their beta cytotoxic effects via a common mechanism, other work suggests that this may not be the case.

Multiple Low Dose Streptozotocin Diabetes

- Diabetes can be induced in mice by repeated injections of sub diabetogenic doses of streptozotocin and was associated with marked pancreatic insulitis, which suggested pathogenic involvement of cell mediated immunity and similarity to human IDDM.
- A further similarity of this model to human IDDM is that the susceptibility to develop diabetes is influenced by genetic factors, as the disease occurs only in certain inbred strains of mice.
- Low dose streptozotocin induced diabetes is a useful tool to study the ways in which the immune processes may augment the effects of beta cytotoxic agent but not the spontaneous development of IDDM.

Dose: - Diabetogenic dose: In Mice: 200mg/kg i.p

Beagle dogs: 15 mg/ kg i.v for three days.

Procedure: -

- Streptozotocin [60 mg/kg body weight] is prepared in citrated buffer [ph 4.5]
- Albino rats of either sex weighing 150-200 g are injected i.p with above solution.
- Animals showing fasting blood glucose levels > 140mg/dl after 48 hours of streptozotocin administration are considered diabetic.
- After six weeks of treatment blood samples are collected from 6 hr fasted animals through caudal vein
- Serum is separated by centrifuge (3000 rpm) under cooling (2-4 °C) for ten minutes.
- Serum glucose level is estimated by glucose- peroxidase method [GOD-POD kit] using autoanalyser.

1.3 Virus induced diabetes

Principle: -

Viruses are one of the etiological agents for IDDM. They produce diabetes mellitus by infecting and destroying beta cells of pancreas.

Various human viruses used for inducing diabetes include RNA picornovirus, encephalomyocarditis [EMC-D], coxsackie B4 [CB-4].

Procedure: -

- 6-8 week old mice are inoculated by 0.1 ml of 1:50 dilutions of D-variant encephalomyocarditis [EMC] through i.p.
- 0.1ml of above dilution contains 50 PFU [plaque forming units] of EMC virus.(mortality due to this concentration of virus is approximately 10-20%)
- A less infecting variant produces a comparable damage by eliciting autoimmune reactivity to the beta cells.
- Infected animals are considered hyperglycemic if there non fasting levels exceed by 250mg/dl the levels of uninfected animals of the same strain.
- Drug samples to be screened are administered orally for a period of 6 weeks.
- Drug samples to be screened are administered orally for a period of 6 weeks.
- After 6 weeks of drug treatment, blood glucose estimation is done to determine the anti diabetic activity.

1.4 Insulin antibodies induced diabetes

Principle: - A transient diabetic syndrome can be induced by injecting gunea pigs with anti-insulin serum. Diabetes persists as long as antibodies are capable of reacting with insulin remaining in the circulation.

Preparation of antibody

Bovine insulin, dissolved in acidified water [ph 3.0] at a dose of 1mg is injected to guinea pigs weighing 300-4oo gm. Anti insulin sera is collected after two weeks of antigenic challenge.

Procedure: -

- Adult albino rats are injected with 0.25-1.0 ml of guinea pig anti- insulin serum.
- Insulin antibodies induce a dose dependent increase of blood glucose level upto 300 mg/ dl.
- The drug sample to be screened is administered by a suitable root and blood glucose level is analysed to determine the activity.

1.5 Hormone induced diabetes

Principle: - Dexamethasone: is a steroid possessing immunosuppresion action, which causes an autoimmune reaction in the islets and produces type 1 diabetes.

Procedure:-

- Adult rats weighing 150-200 gm are injected with dexamethasone at a dose level of 2-5 mg/kg body weight i.p twice a day.
- Repeated injection of same dose level is carried out for a period of 20-30 days resulting in IDDM.
- The sample to be screened is administered through a suitable root.
- Blood glucose is analysed to determine the activity.

1.6 Genetic models

1.6.1 Non obese diabetic mouse [NOD MOUSE]

- NOD mouse is model for IDDM
- Hypoinsulinemia is developed which is caused by autoimmune destruction of pancreatic beta cells in association with autoantibody production.

Procedure:-

- Mice are breed at laboratory by sib mating over 20 generations.
- After 20 generations of sib mating, spontaneous development of IDDM in mice is obtained.Diabetes develops abruptly between 100-200 days of age.[Characterized by weight loss, poly urea, severe glucosurea]
- Animals are treated with the drug sample to be screened.
- Blood sample is analysed for glucose level to determine activity.

1.6.2 Bio breeding rats [BB] rats

BB RATS: The BB rats were discovered in 1974 by Drs Reignald and Clifford Chappel in a commercial rodent breeding company (Biobreeding laboratories Ltd.) in Ottawa. The diabetogenic syndromes of BB rat shares many characteristics with human IDDM. There is genetic predisposition to develop the disease and long prediabetic period followed by abrupt onset of symptoms at around three months of age.

Procedure:-

- Rats are bred at the laboratory by sib mating over 20 generations.
- After 20 generations of sib mating spontaneous development of IDDM in rats is obtained.

- The onset of clinical diabetes is sudden and occurs at 60- 120 days of age. [Clinical presentation is similar to that of humans with marked hyperglycemia, glycosurea and weight loss and decrease plasma insulin, and these results in ketoacidosis if untreated.]
- Animals are treated with drug samples to be screened for a required period of time.
- Blood sample is determined for glucose level to determine activity.

2. Models For NIDDM[2,7,17-23]

2.1 Streptozotocin induced neonatal model for NIDDM

Streptozotocin causes severe pancreatic beta cells destruction, accompanied by decrease in pancreatic insulin stores and rise in plasma insulin levels.

Procedure:-

- Neonatal rats are treated with streptozotocin [90 mg per kg body weight] prepared in citrate buffer [pH 4.5] by i.p at birth or within the first five days following birth.
- After six weeks rats develops symptoms similar to NIDDM.
- Rats showing fasting blood glucose level above 140 mg/ dl are considered diabetic.
- Further steps are similar to alloxan induced diabetes model.
- Drug sample to be screened is administered by a suitable route and blood glucose level is analyzed to determine the activity.

2.2 Adrenaline induced acute hyperglycemia

Adrenaline is a counter regulatory hormone to insulin. It increases the rate of glyconeolysis and the glucose levels in blood causing hyperglycemia.

Procedure:-

- Adult albino rats are injected at a dose level of 0.1 mg / kg through s.c. route
- The dose produces peak hyperglycemic effect after one hour and lasts upto four hours.
- The drug sample to be analysed is administered through a suitable route.
- Blood glucose is determined.(The oral hypoglycemic agents can be screened by this method).

2.3 Dithizone induced diabetes

Principle

Organic agents react with zinc in islets of langerhans causing destruction of islet cells and producing diabetes. Compounds such as dithizone, EDTA, 8-hydroxy quinoline are used to induce spontaneous type 2 diabetes in experimental animals.

Dithizone at dose levels of 40-100mg/ kg (i.v) produces type two diabetes in mice, cats, rabbits and golden hamsters.

Procedure:-

- Adult rabbits weighing 1.8-2 kg are divided into two groups of six animals each.
- An exactly weighed amount of Dithizone is dissolved in dilute ammonia solution (0.2-0.5%)
- The solution is warmed to 60-70 C for 10 minutes to aid solubility of dithizone.
- Dithizone injection at a dose level of 50-200 mg/ kg will produce triphasic glycemic reaction.
- Initial hyperglycemia will be observed after 2h & normoglycemia after 8h, which persist for upto 24 h. permanent hyperglycemia, is observed after 24-72 h.
- The drug sample to be analysed is administered through a suitable route and blood glucose determined.

3 Models For Insulin Sensitivity And Insulin Like Activity[3,6,10,18,20-23]

3.1 Euglycemic clamp technique

Principle:-

This method has proved to be a useful technique of quantifying in vivo insulin sensitivity. A variable glucose infusion is delivered to maintain euglycemia during insulin infusion. The net glucose uptake is quantified and sensitivity of body tissue to insulin determined.

Procedure:-

- Adult albino rats weighing 150-200 g are fasted overnight and anaesthised with pentobarbital (40mg/ kg i.p).
- Catheters are inserted into jugular vein and in femoral vein for blood collection & insulin and glucose infusion respectively.

- To evaluate insulin action under physiological hyperinsulinemia (steady state plasma insulin concentrationduring the clamp test is around 100 U/ dl) and maximal hyper insulenmia, two insulin infusion rates i.e. 6 and 30 U/kg/min are used.
- The blood glucose concentrations are determined from samples collected at 5 min intervals during the 90 min clamp test. The glucose infusion rate is adjusted so as to maintain the basal levels.
- The glucose metabolic clearance is calculated by dividing the glucose infusion rate by steady state blood glucose concentration.
- The drug sample to be analysed is administered through a suitable route and the blood glucose is determined.

3.2 Assay for insulin & insulin like activity

This assay involves comparing two standard solutions of insulin with the test drug for it's insulin like activity

Procedure:-

- Four groups of six rabbits weighing at least 1.8 kg are used
- Two standard solutions of insulin containing one unit and two units respectively and two dilutions of sample whose potency is being examined are prepared.
- As diluent a solution 0.1- 0.25% w/v of either m-cresol or phenol and 1.4 -1.8 w/v of glycerol acidified with hydrochloric acid to a pH between 2.5-3.5 is used.
- Each of the prepared solution (0.5 ml) is injected subcutaneously.
- After one hour and 2.5 h of each injection, a suitable blood sample is taken from the ear vein of each rabbit and blood sugar determined preferably by glucose oxidase method.

DISCUSSION

The above mentioned models have given broad spectrum for the evaluation of the anti-diabetic activity, each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying aetiopathogenic mechanisms that could also operate during the evolution of type 2 diabetes in humans. Hence, care must be taken in interpretation and extrapolation of the results obtained from these animal models to humans. In the screening programme of anti-diabetic compounds, it is particularly important to note that some animal models are better suited to screen particular class of anti-diabetic compounds. Since initial medicinal chemistry campaigns and screening, generally require the testing of many compounds in the industrial research environment, use of smaller animal

models such as mice, will reduce the expense of producing test materials while some advanced efficacy studies or toxicological examinations which require invasive procedures and large blood and tissue samples, may be facilitated by using animals with large body size such as rat or other non rodents. Further, the selection of particular animal model is particularly depending on the investigator's choice particular strain, aim of scientific strategy, type of drug being sought, institutional financial and facility resources in the type 2 diabetes research and pharmaceutical drug discovery and development programme.

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