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EVALUATION OF ANTI DIABETIC ACTIVITY BY VARIOUS SCREENING MODELS – A REVIEW

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ABSTRACT

Diabetes is evolving as one of the most fatal diseases confronting humanity right behind cancer and cardiovascular disease. The number of adults fighting with diabetes in India is projected to ampify threefold, from the existing 19.4 million in 1995 to 57.2 million in 2025. Although insulin therapy is widely used for management of diabetes mellitus, its many side effects such as, insulin resistance, anorexia, brain atrophy and fatty liver after chronic treatment makes it a risky proposition. Therefore, extensive research, which is still at a nascent stage, is requires finding more effective and safer hypoglycemic agents. Before that, when focusing on the area of preclinical studies, more and more advances have been came into existence in research by the help of screening methods. These methods are very useful to evaluate the parameters related to diabetes. In this present review, we made an exposure regarding many screening methods on evaluation of diabetes in the aspects of preclinical studies.

INTRODUCTION:

Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action or both. It is made up of two types: Type I and Type II. Type I diabetes often referred to as juvenile diabetes, is insulin dependent and known to affect only 5% of the diabetic population. The Type II, which is non-insulin dependent, usually develops in adults over the age of 40.

The management of diabetes is a global problem until now and successful treatment is not yet discovered. Currently available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulfonyl ureas, metformin, glucosidase inhibitors, troglitazone, etc. But these are reported to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea. It is currently affecting around 143 million people and the number of those affected is increasing day by day, by 2030 it is predicted to reach 366 million populations worldwide. According to World Health Organization (WHO) projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world. Experimental induction of diabetes mellitus in animal

models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes. Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic considerations. Experimental diabetes mellitus is generally induced in laboratory animals by several methods that include: chemical, surgical and genetic (immunological) manipulations.

Animal Models for Type-1 & Type-2 Diabetes

I- Chemically induced diabetes-

Chemical agents which produce diabetes (diabetogenic agent) can be classified into three categories, and include agents that: specifically damage ß- cell, cause temporary inhibition of insulin production and/ or secretion and diminish the metabolic efficacy of insulin in target tissue. The following text is summarizes the models based on use of diabetogenic agents.

Streptozotocin (STZ) induced diabetes: Streptozotocin (STZ) is a glucosamine-nitrosourea compound that has been in clinical trial since 1967. It was formerly designated streptozotocin, but its name has been shortened by the US Adopted Names Council. A New Drug Application has been filed with the US Food and Drug Administration by The Upjohn Co (Kalamazoo, MI) for marketing under the trade name Zanosar. Approval of this application is imminent. STZ induces diabetes in almost all species. Diabetes can be induced by STZ either by either by single injection of STZ or by multiple low dose injection of STZ. STZ is the most commonly used drug for induction of diabetes in rats .Intra-venous injection of 60mg/kg dose of streptozotocin in adult wistar rats causes swelling of pancreas followed by degeneration of Langerhans islet beta cells and induces experimental diabetes mellitus in the 2-4 days. Three days after degeneration of beta cells, diabetes was induced in all animals. Nicotinamide-adenine dinucleotide (NAD)in pancreas islet beta cells and causes histopathological effects in beta cells which probably intermediates induction of diabetes .While rodents have been extensively used as the animal species, other animals have also been utilized. In a study, the induction of diabetes in New Zealand male rabbits was accomplished by single intravenous injection of streptozotocin (65mg/kg body weight). The study was designed to investigate the biochemical and histomorphological changes occurring due to streptozotocin-induced diabetes mellitus in rabbits .Insulin-mediated glucose metabolism has investigated in streptozotocin (STZ)-treated diabetic pigs to explore if the STZ-diabetic pig can be a suitable model for insulin-resistant, type-2 diabetes mellitus. This study concluded that a slow infusion of STZ (130 mg/kg) in pigs on a low-fat diet induces the characteristic metabolic abnormalities oftype-2 diabetes mellitus and its sensitivity to oral metformin therapy. It is therefore a suitable humanoid animal model for studying different aspects of metabolic changes in type-2 diabetes mellitus. Insulin resistance in STZ diabetic pigs is most likely secondary to hyperglycemia and/or hyperlipidemia and therefore of metabolic origin. Streptozotocin (STZ), preferentially toxic to pancreatic beta cells, is commonly used to model Type-1 diabetes mellitus (DM) in numerous species, including nonhuman primates. A study diabetes mellitus was induced in vervet monkeys (*Chlorocebus aethiops*) by intravenous administration of either 45 (n = 8, STZ-45) or 55 mg/kg STZ (n =12, STZ-55) and ten control (CTL) monkeys received saline. Exogenous insulin requirements increased rapidly for four weeks; STZ-45 insulin dose stabilized

thereafter while STZ-55 doses continued to increase through 16 weeks. Glucose tolerance testing and arginine-stimulated insulin secretion confirmed 80-90% reduction in pancreatic beta cell function in both groups. Body weight was reduced in all STZ monkeys, with return to baseline only in STZ-45at 16 weeks. Elevated blood urea nitrogen and creatinine levels were noted in the STZ-55 group. Alkaline phosphatase also increased with STZ-55 ($p < 0.05$ versus control) whereas STZ-45 alkaline phosphatase elevation was resolved by the end of the study. Red cell parameters were reduced in all STZ monkeys, but more severely in the STZ-55 group. This model demonstrated that diabetes mellitus can be induced and maintained in vervets with a single dose of STZ. The lower dose of STZ (45 mg/kg) significantly improved the toxicity profile without altering efficacy in inducing diabetes mellitus. Finally, sufficient time following induction is recommended to resolve renal, hepatic and hematologic parameters Severe IDDM (insulin-dependent diabetes mellitus) has been produced in the musk shrew (*Suncus murinus, Insectivora*) by a single high dose intraperitoneal injection of 100 mg/kg body weight) of STZ injection. The data of this model indicated that the IDDM in shrew, induced by high doses of STZ, is a unique model characterized by fatty liver and hyperlipidemia and may be useful for studying lipid metabolism of IDDM. Literature also reports STZ induced diabetes cattle, cows in a dose size of 75-150 mg STZ per kilogram of body weight. Alternative dosages and methodologies should be considered in future to induce diabetes in cattle using STZ.

Sucrose-challenged streptozotocin-induced diabetic rat model (STZ-S)

This model screened in vivo antidiabetic activity in sucrose loaded model (SLM) male albino rats. Charles Foster/Wistar strain rats of average body weight 160 ± 20 g weight were used. STZ dissolved in 100 mM citrate buffer, pH 4.5 and calculated amount of the fresh solution was injected intraperitoneally to overnight fasted rats (60mg/kg). Blood glucose levels were checked 48 h later by glucostrips and animals with blood glucose values between144 and 270 mg/dl (8–15 mM) were considered as diabetic. A sucrose load of 2.5 g/kg body weight was given 30min later. Thirty minutes post sucrose load, blood glucose levels were again checked by glucostrips at 30, 60, 90,120, 180, 240, 300 min and at 24 h, respectively. Animals not found diabetic after 24 h post treatment of the test sample were termed as non-responders. The animals, which did not show any fall in blood glucose profile in a group while the others in that group, showed fall in blood glucose profile have also considered as non- responders. Food but not water has withheld from the cages during the experimentation.

Low dose STZ with high fat diet-fed rat model

The model replicates the natural history and metabolic characteristics of human type-2 diabetes and is also suitable for pharmacological screening. The rats are administered high-energy diet of 20% sucrose and 10% lard along with single injection of STZ (30mg/kg body weight). After 4 weeks changes in body weight are recorded and levels of glucose, TG, TC, LDL in serum are analyzed by standard methods. The results suggested that a combination of low dose STZ and high-energy diet intake can effectively induce type-2 diabetes by altering the related gene expressions in major metabolic tissues.

Alloxan induced diabetes

Alloxan is also called as mesozalylurea, mesoxalylcarbamide 2, 4, 5, 6-tetraoxohexa hydropyrimidine orpyimidinetetrone. It is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. Alloxan generates reactive oxygen species in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates

superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defence capacity, and ensure state of insulin-dependent alloxan diabetes. The remarkable discovery of alloxan diabetes came about between a professor of pathology and an apprentice who was foisted on him. The Professor (J. Shaw Dunn) had a lifetime behind him largely dedicated to studies on the kidney and particularly reno-tubular necrosis. The apprentice (McLetchie), despite overburdening duties and discouragement against endocrine research in wartime, developed a passion for endocrine investigation. A Colone Sheehan (later to be enshrined in Sheehan's syndrome) in a brief wartime collaboration with the apprentice left him with a vivid description of hypoglycemia associated with post-partum pituitary necrosis. The apprentice saw this behavior paralleled in rabbits which had been given alloxan in a vague belief that it would further wartime research on the Crush Kidney syndrome, and so, alloxan diabetes was born. The dose of alloxan varies with different species of animals like rat 40-200mg/kg i.v or i.p., mice 50-200 mg/kg i.v or i.p., rabbit 100-150 mg/kg i.v. and for dogs it is 50-75 mg/kg i.v. Alloxan causes triphasic response in animals Stage I-early hyperglycemia of short duration (about 1-4 hr) due to a sudden short lasting decrease or cessation of insulin release and direct glycogenolytic effects on the liver. Stage II-hyperglycemia phase lasting up to 48 hrs and often resulting in convulsion and death. Stage III-chronic diabetic phase consequence of insulin lack histologically only a few ß- cells if any are detectable in animals with fully developed alloxan diabetes. Exogenous insulin readily restores normal blood glucose levels. Another study investigates histopathological abnormalities due to prolonged alloxan-induced diabetes mellitus in rabbits. Diabetes mellitus was experimentally induced in New Zealand white male rabbits by intraperitoneal\ administration of four doses of alloxan as 80 mg/kg body weight at weekly intervals following 12hr fasting .Histomorphological alterations were recorded for pancreas, kidneys, lungs, heart and brain in diabetic rabbits. With the progress of untreated diabetes, the histoanatomical alterations intensified and extended to almost all organs of the body. However, mild changes were observed in gastrointestinal tract with proliferation of yeast in the stomach indicating an increase in the susceptibility of gastric mucosa to yeast cell proliferation. Another investigation reports contrasting effects of alloxan and magnesium on plasma free fatty acids in rats. The study used 28 rats that received alloxan (120mg/kg) intraperitoneally and plasma glucose level measurement after 72hours demonstrated diabetes induction. Analysis of plasma free fatty acids showed a significant increase (751.25mM), compared to the control group (286.68 mM). In contrast, the red blood cell-magnesium level showed a significant decrease from 7.18 mg/dL in control group to 4.89 mg/dL in diabetic rats. The results of the study showed an inverse relationship between plasma free fatty acids and red blood cell- magnesium in diabetic condition. Thus analysis of red blood cell- magnesium upon induction of diabetic condition could provide important information for management of diabetes.

Goldthioglucose obese diabetic mouse model

Type-2 diabetes with obesity can be induced in mice by intraperitonial injection of goldthioglucose (GTG) in a dose of 150-350 or 200 mg/kg. The animal gradually develops obesity, hyperinsulinaemia, hyperglycemia, insulin resistance over a period of 16- 20 weeks after GTG injection. The GTG is transported in particular to the cells of ventromedial hypothalamus and causes necrotic lesions, which subsequently are

responsible for the development of hyperphagia and obesity. It also increases body lipid, hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreases glucose metabolism in muscle, abnormalities that are qualitatively similar to genetically obese mice (*ob/ ob*). In addition, it exhibits many molecular defects in relation to insulin signaling pathways injection.

Atypical antipsychotic-induced diabetic model

Besides the therapeutic improvement over first-generation antipsychotics, the fact that prescription of atypical agents is also associated to the emergence of severe metabolic derangement in patients is not a mystery anymore. These include glucose deregulation, insulin resistance, hyperlipidemia, weight gain and hypertension, which put patients at increased risk of cardiometabolic disorders. The relationship between diabetes and antipsychotic drugs Pelagia Research Library requires a careful analysis. Patients with schizophrenia are known to suffer from diabetes more often than the general population. In this series one study has been investigated the diabetogenic effects of a spectrum of antipsychotics, both atypical and typical. Healthy animals have treated acutely with clozapine (10 mg/kg), olanzapine (3.0 mg/kg), risperidone(1 mg/kg), ziprasidone (3 mg/kg) or haloperidol (0.25 mg/kg) and tested using the hyperinsulinemic-euglycemic and hyperglycemic clamp procedures. Clozapine and olanzapine had a rapid and potent effect on insulin sensitivity by lowering the glucose infusion rate and increasing hepatic glucose production. Clozapine and olanzapine, as well as risperidone, decreased peripheral glucose utilization. Neither ziprasidone nor haloperidol had a significant impact on insulin sensitivity. In the hyperglycemic clamp, clozapine and olanzapine impaired beta cell function as reflected by a decrease in insulin secretion. Results confirm that antipsychotic medications have an immediate impact on metabolic parameters and the various atypical antipsychotics differ in their propensity to acutely induce metabolic side effects.

Miscellaneous chemical diabetogenic animal models

Dithizone induced diabetes model have been used in pharmacological aspects. Organic agents (8-(p-toluenesulfonylamino)quinoline) react with zinc in islets of Langerhans causing destruction of islet cells and producing diabetes. Dithizone injection at a dose level of 50-200 mg/ kg will produce triphasic glycemic reaction. Initial hyperglycemia will be observed after 2h and normoglycemia after 8h, which persists for up to 24 h. Again hyperglycemia is observed after 24-72 h which lasts for longer period of time. Another study describes the effect of sirolimus on cyclosporine -induced pancreatic islet dysfunction in rats. The sirolimus treatment increased blood glucose concentration in a dose-dependent manner. The combined treatment with sirolimus and cyclosporine increased blood glucose concentration, hemoglobin A1C level, HOMA-R [fasting insulin (mU/mL) fasting glucose (mmol/L) /22.5] index and decreased plasma insulin concentration, immunoreactivity of insulin and pancreatic beta islet cell mass compared with rats treated with cyclosporine A. The results of the study demonstrated that sirolimus is diabetogenic and aggravates cyclosporineA-induced pancreatic islet dysfunction. Cyclophosphamide - accelerated model of diabetes has also been reported. Cailleau.*et al* evaluated the role of IL-1βin the cyclophosphamide-accelerated model of diabetes. Non-diabetic male mice injected with 200 mg/kg cyclophosphamide were treated twice weekly with anti-IL-1β Ab. In contrast, only 34% of mice treated with 0.25mg of anti-IL- 1β Ab became diabetic.

II- Surgically induced diabetes:

This method consists of complete or partial pancreatectomy in animals used for the induction of type-1 or type-2diabetes respectively. Historically, the diabetic dog model discovered by Oskar Minkowski through surgical complete pancreatectomy has been considered to be the first animal model of diabetes and is rarely now used for the investigation. Few researchers have employed this model to explore effects of natural products with animal species such as rats, pigs, dogs and primates. However, partial pancreatectomy and/or combination method son animals particularly non rodents are at times utilized in the diabetes investigation for some specific studies as described below.

Duodenal-jejunal by pass non-obese T-2 DM

This model has been shown to reverse type-2 diabetes (T-2 DM) in Goto-Kakizaki rats, a rodent model of non-obeseT-2 DM. Sham operations have been performed in Goto-Kakizaki and non-diabetic Wistar-Kyoto rats. Two weeks post-duodenal-jejunal bypass, oral glucose tolerance was measured and after three weeks insulin-induced signal transduction and glucose disposal was measured in skeletal muscle. The study proved that bypassing of the proximal small intestine does not increase skeletal muscle glucose disposal. The lack of skeletal muscle insulin resistance in Goto-Kakizaki rats questions whether this animal model is adequate to investigate the etiology and treatments for T-2 DM. Additionally, bypassing of the foregut may lead to different findings in other animal models of T-2 DM as well as in T-2 DM patients.

Non obese partial pancreatectomized diabetic animals

Partial pancreatectomy in animals performed as 70 or 90 per cent (usually 90%) dissection of pancreas has been reported in various animal species namely in dogs, pigs, rabbit and also rats. An animal model in which part of the pancreas has made diabetic due to almost total loss of insulin-secreting B cells, while the remainder of the gland remained normal has also been reported. In rabbits, a vascular clamp is placed across the junction of the body and tail of the pancreas, thus occluding the circulation to the tail. Alloxan (200 mg/kg) was injected i.v. and 4 min later dextrose (0.5 g/kg) was given by same route. After 2 min the clamp was removed. 50% of the animals died in the first postoperative week of surgical complications or of alloxan-induced toxicity to the liver and kidneys. The survivors were killed between 4 and 12 weeks after surgery and were not metabolically diabetic. They had virtuallya complete absence of B cells but a normal population of A, D, and PP cells in the head and body of the pancreas.The islets in the tail of the pancreas appeared entirely normal. This model is considered suitable for studying the effects of locally produced insulin on pancreatic exocrine function in metabolically normal animals. The experimental design permits evaluation of the compound effectiveness on both resistance and secretion of insulin. The use of pancreatectomy in combination with chemical agents, such as alloxan and STZ, produces a stable form of diabetes mellitus in animals. The combination therapy reduces the organ damage associated with chemical induction and minimizes the interventions, such as enzyme supplementation, necessary to maintain pancreatectomized animals. Recently, another model on stable form of type-2 diabetes has been introduced by combination of 50per cent partial pancreatectomy along with NAD (350-mg/kg) and STZ (200 mg/ kg) treatment in Balb/c mice. Additionally, VMH dietary obese diabetic rat has been developed by experimental surgical manipulation of genetically normal animals without the reduction in pancreatic beta cell mass, resembling type-2 diabetes, by combining bilateral electrolyte lesion of VMH and feeding the high fat and high sucrose diet to the animal. It is characterized by marked

obesity, hyperinsulinaemia, hypertriglyceridaemia, insulin resistance, impaired glucosetolerance, moderate to severe fasting hyperglycaemia and defective regulation of insulin secretory response despiteextremely high insulin secretory capacity. It is interesting that significant hyperphagia is observed despite increased leptin levels (leptin resistance) in the VMH lesioned rats. Limitations to surgically induced diabetes include high level of technical expertise and adequate surgical room environment, major surgery and high risk of animal infection, adequate post-operative analgesia and antibiotic administration, supplementation with pancreatic enzymes to prevent malabsorption and loss of pancreatic counter regulatory response to hypoglycemia.

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