Chemical Pancreatectomy: An Invaluable Research Tool in Diabetes Mellitus

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Besides traditional treatment of NIDDM by oral hypoglycemic agents research is constantly being carried out on a number of indigenous plants and herbs to find their usefulness in this disease. It is difficult to find volunteers for trial of such indigenous drugs. Hence we have to depend upon animals in which diabetes is produced experimentally. Alloxan is quite effective in producing experimental diabetes primarily by damaging the beta cells which are highly susceptible to its action. We have to observe certain precautions while this drug is being used other wise their will be very high mortality rate in animals under going this experiment. Following article is based on personal experience of the writer in producing such diabetes, which could be of great help for all those who are in search of a new antidiabetic herb which can be included in the diet of non insulin dependent diabetics.
Key words: Chemical pancreatectomy, Alloxan, beta cell, pancreas.

According to various workers experimental diabetes may be either temporary or permanent, which depends upon demonstrable beta cell lesions[2].

Experimental diabetes can be produced by methods which are grouped according to whether, the damage to the islets is primary or secondary. The first group includes total and subtotal pancreatectomy, alloxan diabetes and streptozotocin diabetes which is essentially chemical pancreatectomy with respect to beta cells. The second group includes diabetes produced by anterior pituitary extracts, thyroid feeding and glucose administration. In this group damage to the beta cells is temporary. If the exciting agent is discontinued in early stages, the beta cell changes can be reversed[2]. The total removal of pancreas results in diabetes, and leads to many other undesirable side effects. As a result sub total pancreatactomy is generally done. Sub total pancreatactomy is sometimes not followed by a diabetic state until a period of about 2-3 months. If 5% of the gland is left, diabetes is not so severe[5].

In total pancreatactomy their is sudden total removal of beta cells, whereas in subtotal pancreatectomy there is partial removal of beta cells and secondary damage to the cells that survive[2].

Alloxan diabetes was first reported by Jacob in 1937 cited by Berger[1] (1961), who noticed an initial hyperglycemia and a subsequent hypoglycemia in rabbits. he also postulated an insulin like action of alloxan. It has now been shown hat the hypoglycemic phase is due to the liberation of excessive insulin from the damaged beta cells and toxic effects of alloxan on the liver. In addition to this Dunn Shehan and Neletchi in 1943, also observed necrosis of the central islet cell[7]. Bailey and Brunschwing later announced (1943) that permanent diabetes would be produced by this method. Which is practicable in animals such as rats and rabbits in which pancreas is too diffuse for operative removal. According to Sokoloverora[6] (1959) younger animals have an islet apparatus with greater regeneration capacity and therefore become less severely diabetic.

Alloxan was first synthesized in 1838 by oxidation of uric acid. In 1943 its action on the pancreas was discovered. It exerts a lethal effects unrelated to any action on the kidney. The site of action was traced to be the islet of Langerhan and has provided a research tool which is invaluable in diabetic studies[3]

1NH -------- CO6
2CO -------- CO5
3NH -------- CO4

Structural formulae of Alloxan

The diabetogenic action is due to greater susceptibility of beta cells to alloxan rather than unusual concentration of the drug in the pancreas[2]. It exerts its effects by damaging the beta cell membrane[8] and the essential group with which alloxan reacts may be dithiol group.

The onset of action is almost immediate, even if the protective substances are given only a few minutes after the administration of alloxan, the cytotoxic action on The pancreas is not prevented. The period of time during which alloxan is active in the body is also very short for example if the circulation to the pancreas is interrupted for only 5 minutes following the injection of alloxan the cytotoxic action on the organ is not manifested[3]. Regarding dosage the appropriate I/V diabetogenic dose is 50-70 mg/kg in dogs and 200mg/kg in rabbits. In rats ED 50 and LD 50 are almost identical and both are almost 50mg/kg I/v or 175mg/kg S/C.

Materials and methods

Production of experimental diabetes

For the preparation of alloxan diabetic animals, method used by But 1962 was adopted. healthy animals were selected. Experiments were carried out on four groups of animals each consisting of ten rabbits. No preliminary starvation was employed. Injections of alloxan were given in these groups at interval of 2 weeks each.

On the day of injection animals were weighed, numbered and kept in rabbit boxes. 0.1ml of blood was

obtained from each rabbit, marginal ear vein for the determination of blood sugar which was kept as control.

Five percent alloxan solution was prepared by dissolving five grams of crystals of aloxan moderate (Eastman Kodak Company) in sterile physiological saline and the volume made up to 100 ml. A calculated volume of the freshly prepared alloxan solution in doses of 150mg/kg body weight was injected intravenously with a 26 gauge needle fitted to a 5ml syringe. The injection was made very carefully and slowly. The time period was controlled with the help of stop clock set for period of 5 minutes for Group 2, and for a period of 3 minutes for group 3 and 4. The time was not controlled for the first group. After the alloxan injection the animals were kept under careful observation for a period of 72 hours. 5% glucose solution was administered subcutaneously to all the animals of group. 4. Food and water was supplied freely during this period and the animals were observed for symptoms of hypoglycemia such as lack of activity or convulsions. On the 4th day following the alloxan injection blood sugar determinations were done to determine the severity of diabetes. Animals with blood sugar levels above 200mg/100ml blood were considered diabetic Facilities for catheterisation could not be availed, so urine sugar could not be tested and we had to depend solely on the blood sugar level for The assessment of hyperglycemia. effect of rate of injection of alloxan on mortality rate.

Rate of injection has definite time relation to the development and severity of diabetes and the incidence of death in the animal. Pincus et al[4] (1954). This was also confirmed in our experiments as shown in Fig 1 Tables 1-4.

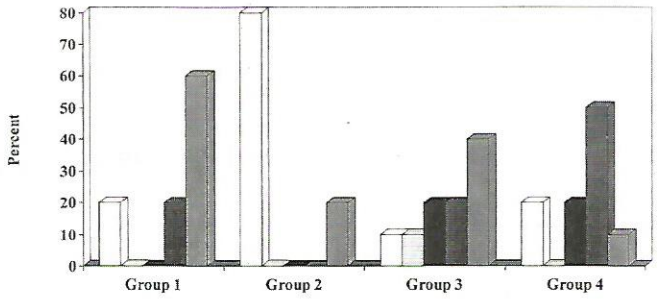

Fig.1. Effect of rate of injection of alloxan on mortality rate and occurrence of diabetes in rabbits

Table-1 Alloxan Diabetes in Rabbits Alloxan 150.0mgs/kg injected intravenously

Animal	Body weight (Kg)	Blood Sugar before injection (mg)	Per100ml after injection
1.	1.09	105	Died
2.	0.90	100	Died
3.	1.20	120.0	320.0
4.	0.80	99	Died
5.	1.20	89	410.0
6.	0.92	103	97.5
7.	1.10	110	Died
8.	0.9	115	110.0
9.	0.6	95.8	Died
10.	1.1	108.8	Died

Table-2 Alloxan Diabetes in Rabbits Alloxan 150.0mgs/kg injected intravenously over a period of five minutes

Animal	Body weight (Kg)	Blood Sugar (mg)	Per 100 ML after injection
1.	1.50	70.0	72.0
2.	1.10	65.5	60.2
3.	0.8	83.5	Died
4.	1.23	92.3	88.5
5.	1.3	91.6	90.5
6.	1.09	90.1	Died
7.	1.0	100.5	95.5
8.	1.2	84.5	80.5
9.	0.75	100.0	95.0
10.	1.25	99.5	90.2

Table-3 Alloxan Diabetes in Rabbits Alloxan 150.0mgs/kg injected intravenously over a period of three minutes

Animal	Body weight (Kg)	Blood Sugar (mg)	Per 100 ML after injection
1.	0.8	107.0	Died
2.	1.10	100.0	150.0
3.	1.50	89.0	468.0*
4.	1.25	105.0	256.0
5.	0.85	102.0	Died
6.	1.20	101.0	280.0
7.	1.09	110.0	Died
8.	1.20	97.0	450.0*
9.	1.10	90.0	250.0
10.	0.80	104.0	Died

*Animals observed in convulsive attacks

Table-4 Alloxan Diabetes in Rabbits Alloxan 150.0mgs/kg injected intravenously over aperiod of three minutes with subcutaneous glucose injection

Animal	Body weight (Kg)	Blood Sugar (mg)	Per 100ml after injection
1.	1.30	85.0	210.0
2.	1.10	100.0	310.0
3.	0.90	110.0	325.0
4.	1.20	100.50	Died
5.	1.23	90.0	250.0
6.	1.12	99.5	310.0
7.	0.85	101.5	100.0
8.	0.95	105.0	340.0
9.	1.10	95.0	90.0
10.	1.20	110.0	305.0

Results and discussion

In group I animals where rates of injection of alloxan was not controlled, mortality rate was very high and six out of the total of ten animals died (60%). Two rabbits 20% developed severe diabetes and the remaining two 20% did not develop diabetes. Table-1 shows their detailed results.

In group-II of 10 animals in which the alloxan injection was given over a period of 5 minutes, only two animals died i.e. mortality rate (20%) and the rest 80% did not develop diabetes. Table-2 shows their detailed results. In group 3 of 10 animals in which alloxan injection was given over a period of 3 minutes. The mortality rats was 40% in this group. Four animals died within 12-18 hours of injection. Two rabbits were observed in convulsive attacks and were given 5% glucose intravenously, which resulted in prompt recovery. Then these animals were freely supplied with food and water. These two more rabbits from this group developed mild diabetes. One was doubtful while one did not develop diabetes. Their results are shown in Table-3.

In group-IV where alloxan injection was given over a period of 3 minutes, they were protected by a subcutaneous injection of 25ml of 5% glucose given 5 hours after the alloxan injection to all the rabbits. Table-4 shows their detailed results. In this group 50% animals i.e. five developed severe diabetes with blood sugar levels above 300mg%. Two rabbits i.e. 20% developed mild diabetes with blood sugar level between 200-300mg. Two rabbits failed to develop diabetes i.e. 20% and one of them died i.e. mortality (10%). The death of this animal was perhaps due to toxic effects of alloxan on other organs like kidney or liver because otherwise this animal was also protected by glucose injection and there was no chance of hypoglycemia.

Experimental diabetes can be produced by injecting a calculated volume of freshly prepared alloxan solution in a dose of 150mg/kg body weight. For best results, the rate of injection should be over a period of 3 minutes and five hours after the alloxan injection 25mls of 5% glucose solution is to be administered subcutaneously to protect from hypoglycemia.

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