Diabetogenic Drugs in the Vervet Monkey

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SUMMARY

Alloxan and streptozotocin were used to cause beta cell lysis in vervet monkeys used as recipient models for pancreatic allografts.

Tests were performed on these animals to evaluate the effect of the drugs on carbohydrate metabolism. Streptozotocin is preferred as the drug of choice in creating a non-pancreatectomised hyperglycaemic recipient for pancreatic allografting.

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The first vascularised allograft was performed on the dog in 1957.[1] Most of the subsequent work in this field has been carried out in this species.[2,3] Kelly *et al.*[4] and Lillehei and others[5] have carried out simultaneous renal and pancreatic allografting on patients with juvenile onset diabetes and diabetic nephropathy. Marks *et al.*[6] have indicated the importance of late islet cell deficiency as a common cause of death in intractable chronic pancreatitis. The final role of vascularised pancreatic allografts remains to be assessed, but initial experience has suggested that the method can be employed to reverse at least some of the effects of the islet deficiency. The anatomy of the pancreas is similar in man and in the other higher primates. The vervet monkey (*Cercopithecus pygerythrus* F. Cuvier) has been chosen as a non-human model because it is readily available in Natal and has been the subject of immunological study in this region. The viability of a pancreatic allograft may be assessed by the capacity of the graft to produce insulin. Total pancreatectomy before grafting is laborious, hazardous and removes all exocrine pancreatic tissue. Selective destruction of the beta cells of the islets of the recipient has therefore been preferred as a method of providing a 'diabetic' model in a small non-human primate.

Two drugs are available for this purpose. The diabetogenic action of alloxan in rats has been reported.[7,8] Streptozotocin has been evaluated in a wider range of animals, including non-human primates.[9-11] Neither has been evaluated in the vervet monkey. This study has been designed to evaluate their diabetogenic action in this species. Plasma glucose levels in fasting animals, urine volumes, pH, and urinary glucose levels were determined, and glucose tolerance tests have been carried out. The glucose tolerance tests were carried out as subdiabetic animals have normal fasting glucose levels but show impaired carbohydrate metabolism when challenged with a glucose load.

MATERIALS AND METHODS

Animals and Diet

Ten healthy adult vervet monkeys of mass 2,0 kg to 5,15 kg were housed in individual metabolic cages. For a month before the tests they were given a standardised diet consisting of approximately 70% carbohydrate and 30% protein. Enough fresh fruit and vegetables were included to ensure an adequate supply of mineral salts and

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vitamins. Such a standardised diet is essential, as the results of the glucose tolerance tests can be influenced by a restricted carbohydrate intake.[12]

Tests on Normal Monkeys

Before the administration of the diabetogenic drugs, daily fasting plasma glucose concentrations were determined. Glucose tolerance tests were performed on the monkeys to establish the normal carbohydrate metabolism. Saline control tests substituting isotonic saline for the glucose load were performed on 2 monkeys.

To establish normal urine values, 24-hour urine volume was noted. Samples were tested for protein, blood and pH, using Hema-Combistix (Ames Laboratories), and for glucose by means of Clinitest tablets (Ames Laboratories), using the 'two-drop' method.[12]

Glucose Tolerance Tests

The glucose tolerance tests were performed in the following manner. After an overnight fast the animals were anaesthetised with an intramuscular injection of 15 mg/kg of ketamine hydrochloride (Ketalar; Parke-Davis). Muscular spasm was counteracted by intravenous administration of 2,5 mg/kg of a benzodiazepin derivative (Valium; Roche). During the preliminary studies it was found that stress before anaesthesia resulted in elevated fasting blood glucose levels and abnormal glucose tolerance curves. Stress was therefore reduced to a minimum by the use of 'crush' metabolic cages which enable the experimenter to quickly immobilise the animals against the front of the cage where they can be easily injected.

A polyethylene catheter was inserted into the femoral vein via the saphenous vein. A control blood sample was taken and 2 000 mg glucose in a 40% solution was injected via the catheter.

The glucose load was not adjusted to the weight of the animal, since this has been found to be unnecessary.[14] Blood samples were taken at 5-min intervals for 30 min, and at 10-min intervals for a further 30 min. The blood samples were taken into tubes containing a few crystals of sodium fluoride and the glucose concentration was determined on a Beckman analyser using the enzyme glucose oxidase.

Diabetogenic Drugs

A mixture of 100 g streptozotocin (Upjohn) and 96 g citric acid was dissolved in sufficient sterile apyrogenic water to make a solution containing 10 mg/ml of streptozotocin. Sodium hydroxide was added to pH 4,5.[13] The solution was immediately injected intravenously into 5 monkeys in a dose of 60 mg/kg.

A 10% solution of alloxan (British Drug Houses) was made up to pH 5 in apyrogenic water. After sterile filtration the alloxan solution was immediately administered by intravenous injection to 5 animals in a dose of 50 mg/kg.

Experimental Procedure

After administration of these drugs to 2 groups of monkeys the following tests were performed: fasting plasma glucose determinations for 4 days and 3 times a week thereafter, 24-hour urine volume monitored as previously described at the preceding intervals mentioned, and glucose tolerance tests on the surviving monkeys after 40 - 45 days.

RESULTS

The daily fasting plasma glucose concentration of the animals treated with alloxan and streptozotocin are shown in Table I. In the alloxan group there was a mean increase of 65 mg/100 ml on day 1, and thereafter the glucose concentration rose by 213 mg/100 ml on day 2 and continued to rise, reaching a peak of 445 mg/100 ml on the 7th day. The concentration then dropped and became stable at 155 and 160 mg/100 ml after day 18. All the animals receiving streptozotocin became hypoglycaemic on day 1 after injection. The mean increase on day 2 was 370 mg/100 ml, after which the glucose concentration dropped. Hyperglycaemic stabilisation occurred after 14 days. There was variation in response to both drugs, but this variation was greater in the group treated with alloxan, as can be seen from the standard deviations. In this group, animal 268 failed to show marked hyperglycaemia (maximum plasma glucose concentration was 150 mg/100 ml on day 7) while monkey 216 died on day 7 with a plasma glucose concentration of 1 135 mg/100 ml. The mean volume of urine excreted in a 24-hour period in normal animals was 215 ml, and there was no glucose, blood or protein present. The pH range was 7 - 8.

TABLE I. DAILY FASTING PLASMA GLUCOSE CONCENTRATIONS (mg/100 ml) OF ANIMALS TREATED WITH ALLOXAN AND STREPTOZOTOCIN

Diabetogenic drug	Monkey	Day 0	1	2	3	4	7	11
Alloxan	**169**	**65**	**206**	**194**	**248**	**342**	**239**	**195**
	193	**69**	**74**	**224**	**318**	**354**	**255**	**290**
	167	**72**	**193**	**456**	**570**	**582***	**—**	**—**
	216	**75**	**112**	**786**	**500**	**648**	**1135***	**—**
	268	**74**	**98**	**86**	**127**	**129**	**150**	**132**
	Mean	**71**	**136**	**349**	**353**	**411**	**445**	**206**
	SD	**4**	**53**	**249**	**162**	**185**	**401**	**65**
Streptozotocin	**212**	**63**	**23**	**405**	**411**	**345**	**270**	**285**
	183	**79**	**19**	**312**	**384**	**276**	**195**	**201**
	262	**75**	**23**	**369**	**354**	**477***	**—**	**—**
	263	**65**	**53**	**485**	**396**	**264**	**340**	**219**
	257	**65**	**50**	**450**	**306**	**225**	**240**	**321**
	Mean	**69**	**34**	**404**	**370**	**317**	**261**	**257**
	SD	**6**	**15**	**61**	**37**	**89**	**53**	**49**

* Animals died.

In the alloxan-treated animals the volume of urine excreted was below normal on day 1 and subsequently increased, reaching a maximum on day 4 with a mean of 298 ml. The animals with a high plasma glucose concentration excreted between 330 and 750 ml on days 3 and 4. The others showed no marked polyuria. The mean urine volume of the animals treated with streptozotocin was well above normal on all days and the maximum was 620 ml on day 3.

In both groups of animals 2 had traces of glucose in the urine on day 1 and all (except alloxan animal 268) exhibited marked glucosuria on the subsequent days. Animal 268, which failed to become hyperglycaemic, excreted between 30 and 80 ml urine per day, and no traces of glucose were present at any stage. A slight amount of blood was excreted by this animal on day 2, while the other animals treated with alloxan showed no trace of haematuria.

Protein was present in the urine of 3 of the alloxan group monkeys on days 1 and 2, and from day 8 onwards. In the streptozotocin group a lot of protein was present between days 7 - 11. Before that traces occurred in the urine of 1 or 2 animals. After day 11 no protein was excreted.

The urine of the animals which received alloxan showed a wide range of pH (from 5 to 8) until day 4, when pH was 7 - 8. The urine was acidic (pH 5 - 6) in all the animals on day 8, after which the pH returned to normal. In the streptozotocin group the pH of the urine was normal except between days 3 and 7 when it became acidic (pH 5).

The results of the glucose tolerance tests are shown in Table II and Fig. 1. The plasma glucose concentration in the normal animals returned to a fasting level within 40 min, and the mean 60-min value was 34 mg/100 ml below the mean fasting concentration, as was that of the saline controls. There was no significant difference between the rate of glucose clearance in the heavy animals (mass>5 kg) and in the lighter animals (mass<3,4 kg), although the plasma glucose concentration in the heavy animals tended to return to fasting level sooner. Five minutes after the administration of the glucose load, the plasma glucose concentration had risen by a mean of 225 mg/100 ml in the normal animals, and by 184 mg/100 ml and 128 mg/100 ml in the streptozotocin and alloxan groups respectively. There was no significant difference between these figures. The mean 60-min value of the streptozotocin group was 85 mg/100 ml above the mean fasting concentration and that of the alloxan group was elevated by 27 mg/100 ml. The glucose tolerance curve of the animals treated with streptozotocin was significantly different from that of the normal animals at all sample times except at the 5-min time. The alloxan group did not differ significantly from the streptozotocin group, and both were significantly different from the normal group after the 20-min sample. Animal 268 showed impaired tolerance, with the 60-min plasma glucose concentration being 88 mg/100 ml above its fasting value.

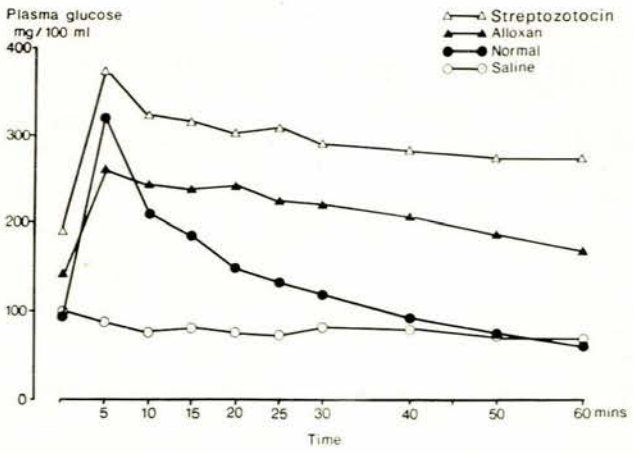

Fig. 1. Mean plasma glucose concentrations in normal, alloxan- and streptozotocin-treated monkeys before and after intravenous infusion of glucose, compared with saline controls.

The results of postmortem examinations which were performed on the animals that died were similar for both groups. All the animals had very little or no subcutaneous fat. The kidneys appeared to be normal, the liver was more friable than normal, and areas of fatty infiltration were visible. No macroscopic changes of the pancreas were visible.

TABLE II. MEAN PLASMA GLUCOSE CONCENTRATIONS (mg/100 ml) IN NORMAL, ALLOXAN- AND STREPTOZOTOCIN-TREATED MONKEYS BEFORE AND AFTER INTRAVENOUS INFUSION OF GLUCOSE, COMPARED WITH SALINE CONTROLS

	Sample times (min)								
	Fast	5	10	15	20	25	30	40	50	60
Saline	**99**	**86**	**75**	**80**	**75**	**73**	**81**	**79**	**72**	**69**
Normal mean	**94**	**319**	**208**	**185**	**148**	**131**	**116**	**92**	**75**	**60**
SD	**18**	**76**	**26**	**22**	**25**	**27**	**31**	**32**	**32**	**26**
Strep. mean	**188***	**372**	**322†**	**315†**	**303***	**307***	**289***	**281***	**273***	**273***
SD	**28**	**92**	**47**	**52**	**45**	**60**	**50**	**60**	**66**	**60**
Alloxan mean	**140**	**268**	**243**	**236**	**240‡**	**225†**	**219†**	**206***	**186***	**167***
SD	**36**	**37**	**40**	**44**	**50**	**43**	**46**	**34**	**26**	**28**

* Significantly different from normal group ($P<0,001$).
† Significantly different from normal group ($P<0,01$).
‡ Significantly different from normal group ($P<0,05$).

DISCUSSION

The hypoglycaemic phase after the administration of streptozotocin is similar to that observed by Junod *et al.*[10] The early hyperglycaemic phase was not noticed, as blood samples were not taken before 24 hours. The triphasic pattern reported by Dunn[15] was not observed, probably because of the sampling times selected in the experiment.

The range of response in the animals treated with streptozotocin was wider than that obtained in rats,[16] but was more constant than that obtained by Pitkin and Reynolds[11] in rhesus monkeys. This variation and that of the animals treated with alloxan, which was greater, are probably due to the differing genetic constitutions of the animals and their adjustment to captivity. These variables are not found in inbred strains of laboratory animals.

Major changes in sensitivity to alloxan due to nutritional status have been reported,[10] but this is an unlikely explanation as all animals were given a standardised diet, and all received alloxan at the same time after an overnight fast.

Streptozotocin appears to produce more reproducible results than alloxan as the range of response was more limited than it was in the animals given alloxan. In the streptozotocin group the hypoglycaemic phase is probably caused by the rapid release of insulin due to permanent damage to the beta cells of the pancreas. The blood glucose reached a maximum on day 2, after which it dropped, becoming stable after 14 days. This suggests that the remaining beta cells were able to overproduce to compensate for the loss of the other insulin-producing cells. As the maximum glucose concentration in the alloxan group only occurred on day 8, it seems likely that the 2 diabetogenic drugs may destroy the beta cells by different mechanisms despite the common end-result.[16]

Contrary to the report that streptozotocin does not cause acidosis in rats,[10] in the present study the urine of the animals became increasingly acidic. This agrees with the findings of Pitkin and Reynolds.[11] There was no difference between the effect of the 2 drugs on the pH of the urine, and renal damage appears to have occurred in both groups, as the animals excreted small amounts of blood and protein.

The reason that the 60-min glucose concentration of the normal animals during the glucose tolerance tests was below the fasting value, is probably that the fasting values were elevated owing to stress. Although this was reduced to a minimum, the animals became excited before being injected, and this would raise the fasting glucose concentration. The elevated 60-min values of the animals treated with alloxan and streptozotocin show that the animals had impaired carbohydrate tolerance. Monkey 268 responded abnormally to the glucose load, emphasising the need for the glucose tolerance test in studies of this nature. The insignificant difference between the results of the heavy and light monkeys supports the view of MacLean and de Wesselow,[14] who found that it was unnecessary to adjust the glucose load.

On the basis of these findings it would appear that streptozotocin is the more satisfactory drug for inducing diabetes in vervet monkeys, but more needs to be known about the mode of action of both drugs in this species. Such investigations are currently being undertaken.

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