Streptozotocin-Induced Experimental Diabetes in Male Wistar Rats

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Abstract. The aim of the present work was to test the sensitivity of young male Wistar rats, Dobrá Voda (Dv:WI) to the diabetogenic effect of streptozotocin (STZ) with regard to their health condition and mortality rates. Eight-week-old rats, weighing from 200 to 230 g, were randomised into five groups of eight animals. Streptozotocin was administered by i.v. injection in doses of 40, 50, 60 and 70 mg/kg body weight. The animals were kept on a standard diet with free access to water for 4 months. The highest STZ dose (70 mg/kg) was lethal to the animals, the doses of 50 and 60 mg/kg induced persistent hyperglycaemia with glucose levels above 20 mM. Body weights of STZ treated rats from all experimental groups were significantly lower than those of control animals. Considerable polyuria was observed in all STZ treated rats. About 40 % of the STZ treated animals were found to develop overt cataract between days 90 and 100. At the end of the experiment, significant albuminuria was observed in the experimental groups administered 50 and 60 mg/kg STZ doses. We conclude that young male Wistar rats, Breeding Facility Dobrá Voda (Dv:WI), Slovakia, treated by a single i.v. STZ dose of 50 or 60 mg/kg developed a persistent disease state characterised by severe hyperglycaemia with major clinical signs of diabetes mellitus.

Key words: Streptozotocin — Experimental diabetes – Male Wistar rats

Introduction

Streptozotocin-induced diabetes in laboratory animals presents an experimental model whose value is in the elucidation of causal relationships related to human diabetes mellitus. In rats, streptozotocin (STZ) induces diabetes when used at doses ranging from 45 to 70 mg/kg (Rakieten et al. 1963; Ar'Rajab and Ahrén 1993). At lower doses, STZ-induced diabetes is not stable, since spontaneous recovery occurs. Long-term studies on pathological changes related to hyperglycaemia require a

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stable model of experimentally induced diabetes. Since different strains of the same animal species may differ in sensitivity to the diabetogenic effect of STZ (Rodrigues et al. 1997), it is important to elucidate in detail the dose-response relationship. The aim of the present work was to test the sensitivity of young male Wistar rats, Breeding Facility Dobrá Voda (Dv:WI), Slovakia, to the diabetogenic effect of STZ given intravenously and over a longer period (up to 4 months) to collect data on behavioural, morphological and biochemical changes in relation to STZ dosing.

Materials and Methods

Test substance

Streptozotocin solution (STZ, Sigma Chemical Corp., Germany) was freshly prepared in 0.1 mol/l citrate buffer, pH 4.5, immediately before use.

Test system

A total of 40 male rats of the Wistar strain were used. The animals were of monitored conventional quality, 8–9 weeks old, weighing 200–230 g and came from the Breeding Facility of the Institute of Experimental Pharmacology (IEP SASc) Dobrá Voda (Slovakia). The animal room was kept under standard conditions. It was air-conditioned with 10 air changes per hour and the environment was continuously monitored for the temperature of 23 ± 1 °C and relative humidity of 40–70 %. The animals were housed in groups of eight in cages of type T4 Velaz (Prague, Czech Republic) with bedding composed of wood shaving (exchanged daily). Tap water and pelleted standard diet KKZ-P-M IEP SASc (Dobrá Voda, Slovakia) were available ad libitum.

Procedure

Prior to dosing, the animals were acclimated to laboratory conditions in the toxicological unit for one week. Animals of 8 per group were used. The rats were randomly assigned to 5 groups. The animals were fasted overnight prior to STZ administration. STZ was administered by i.v. injection via the caudal vein in doses of 40 (group A), 50 (group B), 60 (group C) and 70 mg/kg body weight (group D). The concentration of the application solution was 80 mg STZ/1 ml citrate buffer. Animals of the control group (E) received the same volume of 0.1 M citrate buffer as treated animals. Water and food were available immediately after dosing.

During the experimental period of 120 days the following observations were recorded in each animal: clinical signs (daily), mortality (daily), body weight (weekly), food consumption (weekly), water intake (daily). At a month interval starting on the 12^{th} day after STZ administration, blood was collected from the tail vain into heparinised tubes for plasma glucose determination. Urine was collected monthly from each animal using metabolic cages and tested by test strips Hexaphan Lachema (Brno, Czech Republic) for pH, protein, glucose, urobilinogen, bilirubin and ketone. At the end of the experiment urinary albumin was also determined. Both eyes were examined ophthalmoscopically for the presence of cataract in monthly intervals. Approximately 20 minutes prior to the examination with an ophthalmoscope Meopta (Přerov, Czech Republic) a drop of mydriatic solution (1 % Hemotropin) was applied to both eyes. At the end of the test period, the surviving animals were weighed, killed by cervical translocation and exsanguination of the carotid artery, and necropsied by a veterinary pathologist. Descriptions of all macroscopic abnormalities were recorded. Samples of organs and tissues were collected and fixed in 4 % formaldehyde solution. Organ weight data were recorded and weight ratios determined.

Blood taken from the aorta was examined by the biochemical analyser Encore Chemistry System (Baker Instruments, G.B.) for glucose, cholesterol, alkaline phosphatase, the transaminases ALT and AST, total bilirubin, inorganic phosphorus, urea, calcium, creatinine, total proteins, and albumin Activity of the lysosomal enzyme cathepsin D was determined in organ tissues (Barrett and Heath 1977).

Statistical analysis

All results are expressed as mean ± S D. The data were analysed using Kruskal-Wallis ANOVA followed by the Dunnet's test, Tukey-Kramer multiple comparison test and Fisher's exact test.

Results

A few days after onset of the experiment, depressed motility appeared in all STZ treated animals. Further symptoms observed were piloerection and cachexia. In all STZ treated animals considerable polyuria and polydipsia was also observed. The daily output of urine increased during the first two weeks to 100–145 ml, as compared to control values not exceeding 5 ml, and remained stabilised at this high value throughout the whole 4-month period. Glycosuria and haematuria was detected in diabetic animals from groups B (50 mg/kg) and C (60 mg/kg). At the end of the experiment, significantly increased albuminuria was observed: from the control value of 14.0 ± 4.5 mg albumin per 24 h to 41.7 ± 7.7 and 44.4 ± 19.9 mg albumin per 24 h in groups B and C, respectively.

Days 2 and 3 after STZ treatment proved to be the most critical. All the animals from group D (70 mg/kg), two animals from group C (60 mg/kg) and one animal from group B (50 mg/kg) were found dead within this period.

Compared to the control group, STZ treated animals exhibited significantly lower body weight and slower weight gain in all the experimental groups studied (Table 1). The absolute body weight increase was significantly lower in STZ treated rats, with more distinct values in groups B (50 mg/kg) and C (60 mg/kg).

In all STZ treated groups, food consumption was significantly higher than in control animals at all time points studied (Table 2). Control animals had a rather stable water intake of about 27 g/animal/day, while that of diabetic rats was significantly higher (Table 3).

Table 4 presents the plasma glucose levels measured at monthly intervals. In groups B and C persistent hyperglycaemia with glucose levels over 20 mM was observed during the whole 4-month study period.

Blood biochemistry (Table 5) showed a statistically significant increase of alkaline phosphatase in groups B (50 mg/kg) and C (60 mg/kg), of aminotransferases ALT and AST in group C (60 mg/kg), and of urea in all STZ experimental groups.

In the doses of 40, 50 and 60 mg/kg, STZ caused a significant increase in lysosomal cathepsin D activity in the kidneys and pancreas. In the liver, cathepsin D activity increased after the dose of 60 mg/kg, while in the heart and spleen it remained unchanged at all doses (Table 6).

Table 1. Body weight (g) in STZ treated male rats

Day	A 40 mg/kg $n = 8$	B 50 mg/kg $n = 7$	C 60 mg/kg $n = 6$	E Control $n = 8$
0	211 ± 5	216 ± 6	210 ± 5	212 ± 8
8	228 ± 9*	211 ± 18*	199 ± 12*	254 ± 17
36	248 ± 45*	216 ± 21*	196 ± 19*	328 ± 29
64	297 ± 39*	231 ± 33*	218 ± 29*	377 ± 39
92	319 ± 43*	235 ± 31*	227 ± 27*	412 ± 45
120	322 ± 51*	236 ± 31*	235 ± 28*	423 ± 52

Mean ± S.D. * Significantly different from controls: $p < 0.05$. 1 animal in group B, 2 animals in group C, and all the animals in group D died at the beginning of the experiment.

Table 2. Food consumption (g/1 animal/1 day) in STZ treated male rats

Week	A 40 mg/kg $n = 8$	B 50 mg/kg $n = 7$	C 60 mg/kg $n = 6$	E Control $n = 8$
1	25	26	24	26
2	31	35	37	21
3	30	34	34	18
4	28	39	38	25
5	33	35	36	22
6	31	30	25	16
7	32	32	29	16
8	29	30	26	16
9	37	33	39	25
10	33	37	34	23
11	33	36	33	22
12	36	37	34	24
13	36	36	36	25
14	33	33	36	24
15	35	36	35	23
16	35	37	37	24
17	33	37	35	21
Mean ± S.D.	32 ± 3*	34 ± 3*	33 ± 5*	23 ± 3

* Significantly different from controls: $p < 0.05$. 1 animal in group B, 2 animals in group C, and all the animals in group D died at the beginning of the experiment.

Table 3. Water intake (g/1 animal/1 day) in STZ treated male rats

Week	A 40 mg/kg $n = 8$	B 50 mg/kg $n = 7$	C 60 mg/kg $n = 6$	E Control $n = 8$
1	77	125	121	26
2	89	142	145	25
3	104	166	144	25
4	93	146	138	27
5	104	144	130	25
6	106	141	133	28
7	102	138	131	28
8	106	127	131	27
9	109	135	116	26
10	104	140	112	27
11	98	131	112	27
12	109	158	106	27
13	105	129	111	28
14	98	122	120	28
15	103	120	117	29
16	106	133	123	27
17	96	133	117	26
Mean ± S D	100 ± 8*	137 ± 12*	124 ± 12*	27 ± 1

* Significantly different from controls $p < 0\,05$ 1 animal in group B, 2 animals in group C, and all the animals in group D died at the beginning of the experiment

Table 4. Blood glucose (mmol/l) in STZ treated male rats

Day	A 40 mg/kg $n = 8$	B 50 mg/kg $n = 7$	C 60 mg/kg $n = 6$	E Control $n = 8$
12	10 1 ± 5 2	20 9 ± 6 2*	24 3 ± 6 5*	7 0 ± 0 5
43	18 3 ± 11 4*	27 7 ± 2 8*	29 6 ± 4 0*	6 9 ± 0 9
77	13 0 ± 7 5*	26 6 ± 3 7*	28 6 ± 2 0*	6 8 ± 2 2
118	15 2 ± 7 2*	32 5 ± 4 8*	30 0 ± 3 1*	6 4 ± 0 9

Mean ± S D * Significantly different from controls $p < 0\,05$ 1 animal in group B, 2 animals in group C, and all the animals in group D died at the beginning of the experiment

Between the experimental days 90 and 100, formation of overt cataract was observed in the STZ treated rats However, the incidence of cataract was not dose dependent. group A (3/8, positive findings per number of animals in the group). group B (5/7), group C (1/6). None of the control animals developed ocular abnormalities

Table 5. Blood chemical values in STZ treated male rats on day 120

Blood chemical value	A 40 mg/kg $n = 8$	B 50 mg/kg $n = 7$	C 60 mg/kg $n = 6$	E Control $n = 8$
CHOL (mmol/l)	2 19 ± 0 60	1 90 ± 0 81	2 78 ± 1 11	1 69 ± 0 35
ALP (mkat/l)	3 17 ± 2 18	4 36 ± 1 82*	6 21 ± 2 42*	1 27 ± 0 36
ALT (mkat/l)	0 72 ± 0 21	0 83 ± 0 36	3 96 ± 2 35*	0 69 ± 0 15
AST (mkat/l)	2 21 ± 0 55	1 79 ± 0 51	4 78 ± 1 77*	2 57 ± 0 69
BTBL (mmol/l)	6 6 ± 2 1	6 8 ± 3 1	7 2 ± 1 9	5 1 ± 1 7
P (mmol/l)	3 17 ± 0 62	3 23 ± 0 70	3 36 ± 0 63	2 89 ± 0 67
Urea (mmol/l)	14 8 ± 4 2*	22 7 ± 6 8*	25 8 ± 5 3*	7 3 ± 2 4
Ca (mmol/l)	2 68 ± 0 16	2 41 ± 0 37	2 41 ± 0 23	2 51 ± 0 24
CRE (mmol/l)	74 ± 16	62 ± 15	59 ± 11	72 ± 12
TP (g/l)	68 36 ± 11 77	62 69 ± 15 79	50 68 ± 4 94	61 49 ± 11 85
ALB (g/l)	39 82 ± 5 52	37 65 ± 6 00	31 42 ± 1 65	37 28 ± 5 60

Mean ± S D * Significantly different from controls $p < 0\,05$ CHOL cholesterol, ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, BTBL bilirubin total, P inorganic phosphorus, Ca calcium, CRE creatinine, TP proteins total, ALB albumin 1 animal in group B, 2 animals in group C, and all the animals in group D died at the beginning of the experiment

In animals found dead during the experiment, necropsy findings showed considerable weight loss, bloody efflusion from nasal and genital openings, piloerection and hyperaemia of skin vessels. All parenchymatous organs were strongly hyperaemic. Similar necropsy results were found in regularly killed STZ treated animals. Ileus and enlarged colon were typical findings in animals from groups B (50 mg/kg) and C (60 mg/kg). Organ weight analysis (Table 7) showed a significant relative weight increase of all examined organs in STZ treated rats with exception of lungs in group A.

Discussion

Streptozotocin, similarly as alloxan, is widely used for chemical induction of diabetes in laboratory animals (Bell and Hye 1983) After intravenous administration,

Table 6. Effect of STZ dose on organ activity of lysosomal cathepsin D

Organ	A 40 mg/kg $n = 8$	B 50 mg/kg $n = 7$	C 60 mg/kg $n = 6$	E Control $n = 8$
Heart	1 62 ± 0 05	1 65 ± 0 09	1 81 ± 0 08	1 70 ± 0 04
Liver	1 19 ± 0 05	1 37 ± 0 05	1 59 ± 0 08*	1 28 ± 0 06
Pancreas	2 04 ± 0 31*	2 78 ± 0 22***	3 05 ± 0 36**	1 52 ± 0 14
Spleen	3 50 ± 0 29	3 77 ± 0 24	3 70 ± 0 19	3 89 ± 0 21
Kidneys	3 39 ± 0 09*	3 79 ± 0 08***	3 70 ± 0 12**	3 13 ± 0 08

Mean ± S D Activity of the enzyme is expressed in μg tyrozine/min mg albumin * $p < 0\ 05$, ** $p < 0\ 01$, *** $p < 0\ 001$ 1 animal in group B, 2 animals in group C, and all the animals in group D died at the beginning of the experiment

Table 7. Relative organ weights (g/100 g of body weight) in STZ treated male rats on day 120

Organ	A 40 mg/kg $n = 8$	B 50 mg/kg $n = 7$	C 60 mg/kg $n = 6$	E Control $n = 8$
Heart	0 331 ± 0 060±*	0 395 ± 0 034*	0 366 ± 0 031*	0 252 ± 0 014
Lungs	0 508 ± 0 131	0 616 ± 0 135*	0 636 ± 0 147*	0 369 ± 0 058
Liver	3 411 ± 0 719*	4 485 ± 0 432*	4 599 ± 0 470*	2 362 ± 0 123
Spleen	0 224 ± 0 021*	0 225 ± 0 011*	0 219 ± 0 029*	0 167 ± 0 017
Kidneys	0 876 ± 0 277*	1 128 ± 0 126*	1 106 ± 0 044*	0 516 ± 0 039
Adrenals	0 022 ± 0 006*	0 032 ± 0 007*	0 029 ± 0 004*	0 013 ± 0 001
Testes	1 354 ± 0 299*	1 691 ± 0 210*	1 580 ± 0 347*	0 924 ± 0 121

Mean ± S D * Significantly different from controls $p < 0\ 05$ 1 animal in group B, 2 animals in group C, and all the animals in group D died at the beginning of the experiment

STZ is a highly effective cytotoxic agent for pancreatic B cells. The binding of the drug to its site of action is completed within a short time, the histological and biochemical changes observed after more than 15 min following intravenous injection are secondary changes and not due to a direct effect of STZ (Rerup 1970).

Selection of an appropriate dosage of STZ is a very important issue. Owing to strain differences (Rodrigues et al. 1997), the diabetogenic doses of STZ range from 45 to 70 mg/kg (Rakieten et al. 1963; Ar'Rajab and Ahrén 1993). In our experiment, the STZ dose of 70 mg/kg was lethal for all animals tested. In groups given STZ doses of 50 or 60 mg/kg a stable diabetes with persistent hyperglycaemia over 20 mmol/l of plasma glucose was observed during the 4-month study period. Rather non-homogenous glycaemia values well below 20 mmol/l were observed in group A dosed with 40 mg/kg STZ. Spontaneous recovery of STZ induced diabetes was observed at STZ dose levels 30–40 mg/kg (Ar'Rajab and Ahrén 1993).

After administration of streptozotocin, a characteristic triphasic response in blood glucose was described (Junod et al. 1967). In the first two hours blood glucose rises. This transient hyperglycaemia is due to sudden breakdown of liver glycogen. The second phase, starting at about 6 hours after STZ dosing, is a hypoglycaemic one, which may be severe enough to lead to death. Hypoglycaemia is more pronounced in fasted animals, therefore STZ should be administerd to fed animals to avoid mortalities (Bell and Hye 1983). Administration of 5 % glucose solution during the first 24 hours following STZ injection prevented early mortalities (Suresh Babu and Srinivasan 1997). The third phase, that of permanent hyperglycaemia, begins at about 10 to 12 hours after STZ administration.

In keeping with the findings of Ramanadham et al. (1989), groups given STZ doses of 50 or 60 mg/kg manifested major clinical signs of diabetes mellitus, such as polyphagia, polydipsia, polyuria and body weight reduction. In agreement with literary data (Leuenberger et al. 1971; Dai et al. 1994), cataract formation was observed between the days 90 and 100. Significant albuminuria, a marker of starting kidney damage, occurred at the end of the 4-month test period. Starting renal dysfunction was corroborated by significantly increased activity of the lysosomal cathepsin D in kidney tissue (Table 6), which was in agreement with literary data (Chouinard and Viau 1992).

Day 2 and 3 after STZ administration proved to be most critical for the animals. The animals found dead were cachectic with indigestion, bloody effusion from nasal and genital openings and with strong hyperaemia of all parenchymatous organs. This state seems likely to be due to urea, ammonia and nitrate poisoning. These ions are formed during decomposition of the N-nitrosomethyl urea moiety of streptozotocin (Gunnarsson et al. 1974; Wilson et al. 1984).

We conclude that young male Wistar rats, Breeding Facility Dobra Voda (Dv:WI), Slovakia, treated by a single i.v. STZ dose of 50 or 60 mg/kg developed a persistent disease state characterised by severe hyperglycaemia with major clinical signs of diabetes mellitus.

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