THE OPTIMUM DOSE OF NICOTINAMIDE FOR PROTECTION OF PANCREATIC P-CELLS AGAINST THE CYTOTOXIC EFFECT OF STREPTOZOTOCIN IN ALBINO RAT

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Background: The natural course of Insulin Dependent Diabetes Mellitus is characterized by progressive destruction of insulin producing (3-cells of the pancreas resulting from an autoimmune process. The toxic effect of some p-cells toxins like Streptozotocin (used to produce animal models of 1DDM) has been associated with the oxidative stress due to enhanced DNA repair and NAD depletion in damaged β-cells. This activity of Streptozotocin has been prevented with the use of nicotinamide. Methods: A light microscopic study was designed to determine the optimum dose of nicotinamide required for protection of pancreatic β-cells against the toxicity of Streptozotocin. 35 adult male albino rats were divided into five equal groups A, L>. C. D and E. the duration of study was 14 days. The animals in experimental groups C, D and E received a single intraperitoneal! injection of nicotinamide 250 mg/Kg, 350 mg/Kg and 500 mg/Kg respectively on day one.

Animals in group A and B acted as normal control and diabetic control respectively. All the animals except those in group A received simultaneous injection of Streptozotocin 32 mg/Kg body weight intraperitoneally in a single dose. Fasting blood glucose was assessed and the animals weighed before starting the treatment, after 48 hours and at the end of the experimental period. Histological studies were carried out at the end of the study period. Results: The blood glucose level and the final body weight of the animals in group C matched the values in diabetic control. Histologically the pancreas had generally reduced p-cells mass ($P < 0.001$) with altered morphology. The animals in group D showed impaired glucose tolerance at 48 hours but were normoglycaemic at the end of the study period. There was some loss of p-cells but a significant number of these cells ($P < 0.05$) showing normal morphology were saved. The animals in group E had normal number of p-cells having normal morphological features. The final body weight and fasting blood glucose of these animals matched the values in normal control (group A). Conclusions: These data suggest that the optimum dose of nicotinamide in regard to prevention against the p-cytotoxic effect of Streptozotocin in albino rat is 500 mg/Kg body weight.

INTRODUCTION

The natural course of IDDM (Insulin Dependent Diabetes Mellitus) is characterized by progressive destruction of insulin producing P-cells of the pancreas resulting from an autoimmune process.

The toxic effect of some P-cells toxins like Streptozotocin (used to produce animal models of IDDM) has been associated with the oxidative stress due to enhanced DNA repair and NAD depletion in damaged p-cells2. This activity of Streptozotocin has been prevented with the use of nicotinamide[3,4,5.]

Nicotinamide is an essential part of two important coenzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide dinucleotide phosphate) and hence it is present in every living cell [6].

It has been suggested that the Diabetogenic effect of Streptozotocin might be due to a reduction in synthesis, an increase in destruction or an increase in metabolic requirement for NAD and the antagonistic effect of nicotinamide could reside in its role as a precursor to NAD synthesis[7].

It is hypothesized that the antagonistic effect of nicotinic-amide is based on the ability of this vitamin to keep p-cell NAD levels above the critical point required for Streptozotocin to effect irreversible P-cell damage[8].

The present study was designed to determine the optimum dose of nicotinamide for protecting the pancreatic p-cells in albino rat.

MATERIALS AND METHODS

Thirty-five adult male albino rats of JPMC strain I weighing 250-300 G were used in this study. They were originally obtained from Charles River, j Breeding Laboratory, Brooklyn, Massachusetts, U.S.A. and were cross bred at the animal house of Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan. All the animals were screened for pre-existing diabetes and the grouping was done according to Table-1.

The animals were weighed and their PBS (fasting blood sugar) done before starting the treatment FBS was repeated after 48 hours and at the end of the study period (i.e. 2 weeks post treatment).

Glucosticks of glucometer (Exatech Company) were used for testing the FBS. A drop of blood was obtained from the tip of the tail by disposable lancents.

Table-1: Groups and treatments

Groups	No. of Rats	Treatment	Dose
A (Normal control)	07	Buffer (Sodium citrate)	0.2 ml/100 g
B (Diabetic control)	07	Streptozotocin	32 mg/Kg
C (Experimental)	07	Streptozotocin + Nicotinamide	32 mg/Kg 1 250 mg/Kg
D (Experimental)	07	Streptozotocin + Nicotinamide	32 mg/Kg + 350 mg/Kg
E (Experimental)	07	Streptozotocin + Nicotinamide	32 mg/Kg + 500 mg/Kg

Streptozotocin purchased from Sigma Chemical Company, Dorset UK was injected as a single intraperitoneal dose in citrate buffer pH 4.5 within 5 minutes after it was in solution. The volume was adjusted so that each animal received the equivalent of 0.2 ml/100 G body weight.

Nicotinamide powder, supplied by Abbott Laboratories, Karachi, Pakistan dissolved in 1 ml distilled water was also injected as a single intraperitoneal dose simultaneously with Streptozotocin.

Feeding was resumed immediately after treatment and the animals were supplied with 5% dextrose solution in drinking bottles to prevent hypoglycemia.

The animals were kept on normal laboratory diet and were killed by ether overdose at 2 weeks post treatment. They were dissected to remove the pancreas. The tissue was fixed in formal saline for 48 hours. Tissue pieces from the splenic end of the pancreas were then processed for paraffin embedding, sectioned serially at 3 pm and mounted on gelatinized glass slides. The sections were stained with H&E and chrome alum haematoxylin and phloxin[9,10].

The islets of Langerhans were studied under light microscope for the following:

1. Total number of β-cells /islets.
2. Morphology of β-cells (cytoplasmic and nuclear details).
3. Nuclear diameter of β-cells (a mean of 100 random observations per animal was taken).

Nuclear diameter of P-cells was measured with the help of an ocular micrometer. Results were recorded as Mean ± SEM. Student's 't' test was done for statistical analysis. Paired 't' test was used to compare the blood glucose levels within groups at variable time intervals[11].

RESULTS

All the animals survived the study period. The animals in experimental group C showed a significant decrease in their body weight which was comparable to that in diabetic control (group B) (Table 2). Animals in both the groups (B&C) appeared ill from the second day following treatment.
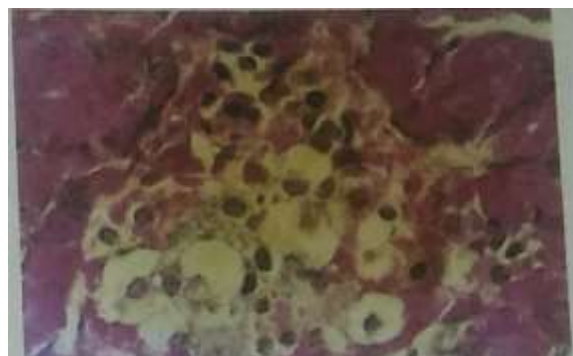

Figure I: Photomicrograph of pancreas from group C showing reduced number of p-cells. clumping of cytoplasm and pyknotic nuclei. X 1000.

Figure 2: Photomicrograph of pancreas from group E showing an islet of Langerhans with normal number of p-cells arranged centrally \ h intact cytoplasmic and nuclear morphology. X 500

The animals in experimental group D showed a gain in their body weight but this was not significant statistically. Their general condition however appeared to be satisfactory.

The rats in experimental group E remained active and healthy looking throughout the study period and showed a significant gain in their body weight which was comparable to that in group A (normal control) (Table 2).

The animals in the experimental group C became diabetic and remained so i.e. their FBS remained elevated till the end of the study period like that of the diabetic control (Group B) (Table 3).

Rats in group D showed hyperglycemia at 48 hours. However, frank diabetes did not develop in these animals and they became normoglycaemic at the end of the study period. The FBS in group E animals

showed a⁻ highly significant increase at 48 hours but this was.

within normal limits and at the end of the study period these animals were normoglycaemic, although their FBS was higher when compared with that of group A at this stage.

A highly significant decrease in total number of 0-cells/islet was observed in group C. the cytoplasm of residual 0-cells was reduced in amount, showed vacuolations and at some places clumping of cytoplasm was also noted. The islets in general appeared empty because of loss of 0-cells as 0-cells make up about 70% of the total population of islets[13] (Figure 1).

The nuclei of the remaining p-cells stained densely, had irregular boundaries and some of them were pyknotic.

A highly significant decrease in p-cell nuclear diameter was also observed in these rats.

All these observations were in every aspect compare to to those present in group B (diabetic control).

In group D animals there was a significant decrease in total number of p-cells (Table 4) but a significant number of P-cells were found to be intact showing normal histology.

In group E the total number of (5-cells/islet was well maintained. They occupied the centre of the islets of Langerhans without any vacuolations or clumping of cytoplasm. The nuclei were round to oval in shape, located in the centre of the cells, stained deep blue and showed prominent nucleoli (Figure 2). In short all the values were maintained close to those of group A except the diameter of β- cells which showed a significant increase Table 4.

Table-2: Comparison of body weight within different groups (Mean ±SEM)

Group	Initial weight (g)	Final weight (g)	Difference (g)	Gain or Loss	Statistical significance
A	269.00 ±4.57	288.50 ±4.71	19.50 ±0.50	Gain	P < 0.05
B	277.00 ±4.87	260.00 ± 5.62	16.50 ± 1.30	Loss	P < 0.05
C	266.18 ±3.92	251.82 ±4.68	15.00 ± 1.23	Loss	P < 0.05
D	268.00 ± 4.25	272.00 ± 4.67	4.50 ±0.50	Gain	N.S.
E	276.12 ±3.67	296.75 ± 3.67	20.63 ± 0.97	Gain	P < 0.05

Table-3a: Comparison of difference in fasting blood glucose concentration (mg/di) within groups at variable intervals (Mean ±SEM)

Groups	Initial	At 48 hours	Difference	Statistical significance	Final	Difference	Statistical significance
A	62.25*1.57	67.50±2.52	5.25±2 11	N.S.	67.00±2.62	4.75±5.07	N.S.
B	62.37*3.53	240.00±8.66	177 62±9.00	P < 0.001	326.38±112.24	86.38±10.61	P < 0.001
C	63.84*4.23	231.92±7.88	188.08±8.54	P < 0.001	340.69±11.35	108.77±13.42	P < 0.001
D	65.32*3.23	198.24±6.52	132.92±7.52	P < 0.001	68 46*4.48	121.78±11.32	P C 0.001
E	61.25*2.37	80.00±2.25	18.75±3.28	P < 0.001	83.52	3.52±2.11	N.S.

Table-3b: Statistical analysis of the comparison of fasting blood glucose concentration at variable time intervals between different groups

	At 48 hours	At 2 hours	
A VS B	P < 00.1	P<0.001
A VSC	P<00.1	P<0.001
A VS D	P<00.1	N.S
A VSE	P < 00.1	P<0.001
B VSC	N.S.	NS
B VS D	P < 00.1	P<0.001
B VS E	P < 00.1	P<0.0019
C VS D	P < 00.1	P<0.001
C VS E	P < 00.1	P<0.001
D VS E	P < 00.1	P< 0.001 1

Table-4: Histological data (Mean ±SEM)

Parameter	A	B	C	D	E
No. of β-cells per islets	97.63±2.20	65.44[***]± 3.68	60.28±34.23	80.82 ± 3.44	98.59 ±2.07
Nuclear diameter of β-cells	3.71 ±0.01	3.61[***]± 0.01	3.62 ± 0.01	3.72 ± 0.01	3.74 ± 0.02

*** *Highly Significant (p<0.001)*   * *Significant (P < 0.05)*

DISCUSSION

Streptozotocin is | cytotoxic by its alkylating action on DNA[13] [N]. As part of the excision repair process to remove the alkylated lesions, the nuclear enzyme poly (ADP ribose) synthetize is activated. This enzyme forms poly (ADP ribose) with NAD as a substrate. In the (3-cells this enzyme has been hypothesized to become activated to such an extent that NAD becomes critically depleted resulting in inhibition of proinsulin synthesis and cessation of cellular function leading to cell death[15-16].

The rapid and marked depletion of cellular NAD has been regarded as the primary molecular mechanism behind the destruction of P-cells[17]. Therefore, NAD precursor, nicotinamide has been used successfully by many investigators to prevent streptozotocin diabetes.

Different doses of this vitamin have been used in different animal studies. Dulin and Wyse[18] have used 250 mg/Kg with beneficial effect in streptozotocin diabetes.

The present study was conducted to determine the dose of nicotinamide providing optimum protection against the (3 cytotoxicity of streptozotocin.

The results of our study reveal that nicotinamide at the dose of 250 mg/Kg gave no protection to p-cells. All the rats who received this dose of nicotinamide became diabetic at 48 hours and remained so till the termination of the study period. Their pancreas showed a highly significant decrease in the number of p-cells per islet. The residual P-cells showed cytoplasmic and nuclear changes which were comparable to those of diabetic control.

These results, however, are not in agreement with those of Dulin and Wyse who have shown good protection of p-cells at the dose of 250 mg/Kg.

The rats who received nicotinamide 350 mg/Kg showed impaired glucose tolerance at 48 hours. This appeared to be transitory as all these animals were normoglycaemic at the end of the study period.

The morphology of the P-cells was intact in these animals. However, there was a significant decrease in the total number of p-cells. As substantial number of p-cells with normal morphology was saved, these animals did not develop frank diabetes. Overt diabetes appears only when most of the P-cells have been destroyed[16].

The results are suggestive of a partial protection of P-cells by nicotinamide 350 mg/Kg. Our results again are not in accordance with those of Boutx et al. who have shown complete protection of P-cells by nicotinamide 350 mg/Kg in mice. This suggest that the protective dose of nicotinamide against p-cytotoxicity of streptozotocin is perhaps species related.

The present study the morphology of P- cells in animals who received nicotinamide 500 mg/Kg appeared to be normal. The cytoplasmic and nuclear features remained intact and the total number of p-cells/islet was well maintained. The histologic appearance was consistent with blood glucose levels and general condition of the animals suggesting a complete protection of P-cells by nicotinamide 500 mg/Kg body weight.

It is concluded that optimum protection of p- cells against the toxicity of streptozotocin in albino rat is achieved by nicotinamide 500 mg/Kg body weight.

ACKNOWLEDGEMENTS

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