

The effect of L-Arginine and Insulin on Histological Changes in Streptozotocin-treated Rat Adrenal Gland

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ABSTRACT

Aims: To evaluate the protective role of L-arginine (L-arg) on the morphology of adrenal cortex of streptozotocin (STZ) treated adrenal glands of albino rats. Serum adrenocorticotropin (ACTH) and corticosterone levels were also determined as stress markers.

Study design: Prospective experimental study.

Place and duration of study: This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), JPMC, Karachi, from February to March, 2011.

Material & methods: In a 6 weeks study, 30 male albino rats were divided into 3 groups, containing 10 animals each. Gp-A was treated as control. Gps-B received STZ 37mg/kg intraperitoneally (I/P) only once at the start of experiment. Gps-C received L-arg orally in a dose of 0.3 mg/100GB.W/day a week before STZ treatment. Serum ACTH and corticosterone levels were also measured.

Results: Haematoxylin and Eosin (H&E) stained sections of cortex of adrenal glands of STZ treated Gp-B showed marked pathology and dis-arrangement of cells in all the three zones of adrenal cortex. Atrophy of zona glomerulosa (Z.G.) and hypertrophy of zona fasciculata (Z.F.) was observed in Gp-B along with enhanced secretions of serum ACTH and cortisol. L-arg had moderately reduced the severity of damage to the adrenal cortex in Gps-C there was also a moderate increase observed in the serum levels of ACTH and decreased corticosterone.

Conclusions: L-arg as a nitric oxide (NO) donor and as an antioxidant, plays a significant role in preserving adrenal morphology and functions in cancer patients who are treated with chemotherapeutic drugs like STZ.

Keywords: Streptozotocin, Adrenal Cortex, L-arginine, Insulin, Adrenocorticotropic Hormone

INTRODUCTION

The adrenal gland is an essential stress responsive organ that is part of both HPA axis and sympathoadrenomedullary system.¹ It is chiefly responsible for releasing hormones in response to stress. Factors affecting adrenal gland and causing adrenal insufficiency are either genetic, physical stress induced or induced by chemotherapeutic drugs like mitotane, tanol, cisplatin or STZ.

STZ is a naturally occurring nitrosurea used in cancer chemotherapy². It is used in metastatic carcinoid tumours, Hodgkin's disease and treatment of advanced islet cell carcinoma³. The effect of STZ on different organs has been extensively studied. It is diabetogenic, hepatotoxic, nephrotoxic, and also causes gastric ulceration^{4,3}.

STZ is a pancreatic β -cell toxin which induces rapid and irreversible necrosis of these cells. The mechanism of STZ induced β -cell injury involves excessive reactive oxygen species (ROS) production, lipid peroxidation, protein oxidation and DNA damage leading to β -cell death⁵. Formation of ROS is thought to be a mediator of cytotoxic actions of STZ, leading to oxidative stress⁶. Oxidative stress may be one of the stresses influencing the regulation of HPA axis⁷.

A number of research laboratories have demonstrated that hyper-activation of HPA axis occurs in several animal models of STZ-induced hyperglycemia⁸ when a threat to homeostasis is perceived, HPA axis is activated as an important regulatory mechanism of the stress response. HPA axis activity is initiated by the secretion of ACTH from the pituitary, which then stimulates the secretion of glucocorticoids from the adrenal glands.

Most previous studies have shown that in rodents, STZ-induced hyperglycemia results in a reduced response to insulin, despite increased numbers of insulin receptors.⁹ It has been hypothesized that hyperactivity of HPA axis may play a central role in the pathogenesis of insulin resistance¹⁰. Studies in animal models of STZ-induced hyperglycemia indicate that antioxidants improve insulin sensitivity¹¹.

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L-arg is the substrate for the synthesis of NO and it has direct anti-oxidant activity¹². It is an essential amino-acid which participates in many important biochemical reactions associated with normal physiology of the organism. Exogenous L-arg increases NO production in a variety of cells. It is both a NO precursor and donor¹³. Previous studies have demonstrated that endogenously generated NO is involved in the modulation of corticosterone production and that adrenal NO synthase activity is dependent on extracellular L-arg¹⁴.

MATERIAL AND METHODS

This study was conducted in the Department of Anatomy, BMSI, JPMC, Karachi, for a period of 6 weeks. In this study, 30 healthy male albino rats, 90-120 days old, weighing around 250-300 G, were obtained from the Animal House of BMSI and divided into 3 groups, each containing 10 animals. All the animals were kept under observation for one week prior to the commencement of study, for the assessment of their health status. All the animals were marked by ear punching and weighed. They were kept in propylene cages, equipped with drinking water bottle and wood chip floor bedding under natural environment. Food and water were supplied ad libitum.

Group-A was taken as control. Groups-B and C animals were fasted overnight and administered STZ I/P in a dose of 37mg/kg¹⁵ dissolved in freshly prepared 1ml of Citrate buffer at 4 pH only on the first day of the experiment. Group-C received L-arg orally in a dose of 0.3 mg/100 G B.W/day¹⁶ dissolved in 5cc of distilled water, one week before administering STZ. The serum glucose of the rats was measured at the start of the experiment and then twice weekly by glucose oxidase method from the tail vein by using a glucometer.

The animals were weighed and sacrificed at the end of their respective treatment by using ether anaesthesia. Their abdomen was opened by midline incision and blood was withdrawn by intra-cardiac puncture into polypropylene tubes for ACTH and

corticosterone levels determination by ELISA kits. The adrenal glands were exposed and carefully dissected and were then fixed in buffered neutral formalin for 24 hours. They were later kept in 70% alcohol overnight. Dehydration was done with ascending strengths of alcohol, they were then cleared in xylene and infiltrated with paraffin at 59 degrees. Paraffin blocks of tissue were made and 5 micron thick longitudinal sections were cut by a rotatory microtome. Sections were mounted on labeled glass slides and stained with H&E¹⁷ for a detailed morphological examination of the adrenal cortex.

RESULTS

In group A control is given in (fig 1) with normal morphology of adrenal cortex. Serum ACTH was 130 ± 2.10 pg/ml and corticosterone was 17.0 ± 0.70 which were normal along with the normal glucose levels 121.3 ± 4.4 mg/dl. (Table 1)

In group-B, the Z.F. of STZ treated group under the light microscope was roughly twice the size of the control group (Fig 2), whereas the Z.G. showed shrinkage in size. Moreover, in group-B, there was a severe distortion and dis-arrangement of cells in all the three zones in the form of cytoplasmic vacuolation, pyknotic nuclei, hypertrophy of cells and apparent increase in lipid droplets (Fig. 2).

Serum ACTH was increased to $310 \pm 1.28^{**}$ which is highly significant and corticosterone was significantly increased to $20.8 \pm 1.06^*$ with blood glucose of 434 ± 10.21 mg/dl significantly increased. (Table 1)

In group C, L-arginine treated examination of H&E stained slides revealed a moderate decrease in cortical thickness and a significant decline in cytoplasmic vacuolation (Fig 3). The dilatation of blood vessels had also markedly decreased (Fig. 3). Serum ACTH was 138 ± 1.9 and corticosterone 18.5 ± 0.80 along with glucose $250 \pm 24.73^*$ significantly high. The serum glucose levels were reduced. The serum ACTH and corticosterone levels were near to those of control group-A (Table-1).

Table 1: Change in mean serum ACTH and corticosterone concentration in all 3 groups of Rats

Groups	Treatment Received	Serum ACTH(Pg/ml)	Serum corticosterone(mg/ml)	Glucose (mg/dl)
Group A	Citrate buffer	130 ± 2.10	17.0 ± 0.70	121.3 ± 4.1
Group B	STZ	$310 \pm 1.28^{**}$	$20.8 \pm 1.06^*$	$434.0 \pm 10.21^*$
Group C	STZ + Larginine	138 ± 1.9	18.5 ± 0.80	$250 \pm 24.73^*$

Values are expressed as mean \pm SEM of 15 rats in each group.

*Significant P < 0.01

**Highly Significant P < 0.05

Compared to control.

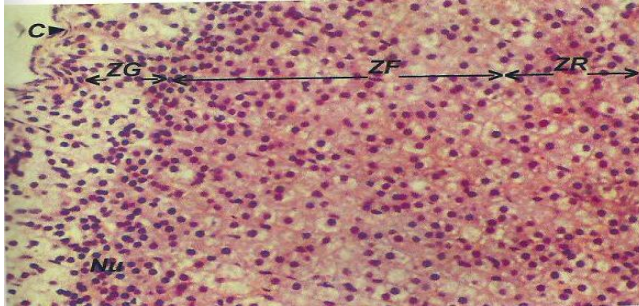


Fig. 1: Photomicrography of 5 microns thick H and E stained section from cortex of adrenal gland in group A control showing capsule (C), zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR)

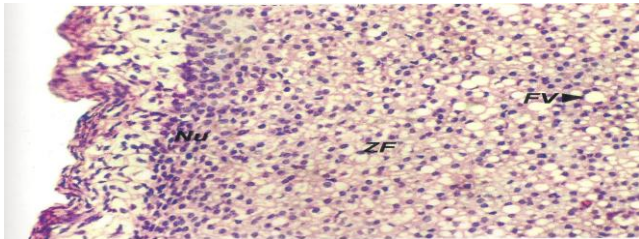


Fig 2: Photomicrography of 5 micron thick H and E stained section from Cortex of adrenal gland in group B (STZ) treated rat showing zona fasciculata roughly twice the size of control group with cytoplasmic vacuolation FV and increased lipid droplet nuclei (NU) closely packed pyknotic.

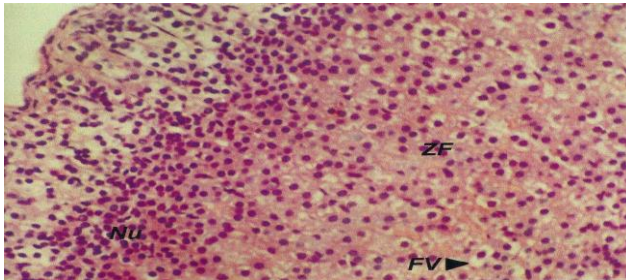


Fig. 3: Photomicrography of 5 micron thick, H and E stained section from the cortex of adrenal gland in group C STZ plus L-arginine treated rat showing scanty fat vacuoles and less closely packed nuclei in zona fasciculata showing restored cortex.

DISCUSSION

The adrenal gland is reported to be the most common endocrine organ associated with chemically induced lesions¹⁸. Adrenal cortical cells contain large stores of lipids used as substrate for steroidogenesis. Many compounds that are toxic for the adrenal cortex are lipophilic and accumulate in these lipid rich cells. Impaired steroidogenesis is a common toxin-induced change that can result in excess steroid precursors and cytoplasmic vacuolation¹⁹.

Many studies have addressed the long term consequences of high glucose levels on the morphology and function of different cell types²⁰. In

particular, adrenal cells subjected to long term glucose treatment exhibit altered oxidative stress parameters, including increased reactive oxygen species (ROS) generation.²¹ Increased activity of the HPA axis, resulting in enhanced ACTH and serum glucocorticoid levels has been described in animal models of STZ induced hyperglycaemia²².

Many reports demonstrate the inhibitory effect of exogenous NO on the steroidogenic pathway. L-arg significantly decreased corticosterone biosynthesis in rat adrenal Z.F. cells. In our study hypertrophy of Z.F. was observed along with an increase in plasma ACTH and corticosterone secretion in STZ treated group. This observation is in agreement with the study conducted by Rebuffat et al in 1988²⁴ who reported that with an intact HPA axis, STZ causes hypertrophy of cells of Z.F. and a rise in plasma corticosterone concentration.

Enlargement of the adrenal cortex has been reported after several types of chronic stress. Both adrenal hypertrophy and hyperplasia have been reported during STZ induced diabetes. Repeated exposure to elevated plasma ACTH during chemical stress may stimulate Z.F. growth and cause Z.G. atrophy. It has been reported that STZ induced hyperglycemia leads to progressive insulin resistance of the peripheral tissues. When glucose and free fatty acids increase, they cause oxidative stress along with activation of stress sensitive signaling pathways. Activation of these pathways, in turn, worsens both insulin secretion and action, leading to insulin resistance²⁵.

Several studies have shown that NO plays a key role in mediating metabolic effects of insulin, including muscle glucose uptake. Long term L-arg treatment as an antioxidant improved peripheral and hepatic insulin sensitivity in type-2 diabetic patients²⁶ and NO derived from NO donors inhibited aldosterone synthesis in adrenal Z.G. cells in bovine, rat and human adrenal glands. This mechanism of inhibition occurred primarily via direct interaction of NO with cytochrome P450 enzymes of steroidogenic pathways. L-arg administration significantly decreased corticosteroid biosynthesis in rat adrenal Z.F. cells²⁷.

Diabetogenic effects of STZ produces diabetic stress which is characterized by decreased body weight and parallel increase in organ weight²⁸. Adrenal size and width of the adrenal cortex increases with chronic activation of HPA axis. Increased adrenal weight, detected in our diabetic rats, could also reflect a prolonged exposure to ACTH concentration. In a study conducted by Kohli R in 2003, treatment of albino rats with L-arg reduced the body weight loss in STZ treated rats. This is in agreement with our study. He further demonstrated

that untreated diabetic rats exhibited low levels of plasma insulin and L-arginine which was restored with L-arg supplementation²⁹.

An important finding of our study was that L-arg supplementation ameliorated weight loss in STZ treated rats. The ability of L-arg to reduce weight loss may result from increase in skeletal muscle protein synthesis or reduction in skeletal muscle protein degradation.

CONCLUSION

L-arg as a nitric oxide donor and as a antioxidant, plays a significant role along with Ins in preserving adrenal morphology and functions in cancer patients who are treated with chemotherapeutic drugs like STZ.

REFERENCES

1. Yvonne M, Ulrich L, Helmer F, Michelle M, Dennis C, William C. Chronic stress induces adrenal hyperplasia and hypertrophy in a sub-region specific manner. *AJP Endo* 2006; 291(5) E965-E973.
2. Etuk EU (2010) Animal model for studying diabetes mellitus, Agriculture and Biology. *J. North Am*; Vol I, Issue 2, pp 130-34.
3. Debro ME and Porter GA (2008) Clinical nephrotoxins. 3rd edition, Springer Publications, USA, pp 990.
4. Piyachaturawat P, Poprasit J, Glinsukoon T. Gastric mucosal sections and lesions by different jobs of STZ in rats. *Toxicol Let* 1990; 55: 21-29.
5. Mariyah Hidayat, Muhammad Faseeh Nisar, Sheema Farhan Akram, Muhammad Akram Zahid. Effect of STZ on the morphology of proximal convoluted tubules in albino rats. *Pak J. Med Health Sci* 2012; 6(2): 298.
6. Haider Raza, Annie John. Streptozotocin induced cytotoxicity, oxidative stress and mitochondrial dysfunction in human hepatoma Hep G2 cells. *Int J Mol Sci* 2012; 13(5): 5751-67.
7. Koichi Asaba. Attenuation by reactive oxygen species of glucocorticoid: Suppression on proopiomelanocortin gene expression in pituitary corticotroph cells. *Endocrinology* 2003, Brief communication.
8. Berrak C. Yegen. Oxytocin and HPA axis. *Marmara Pharmaceutical Journal* 2010; 14: 61-66.
9. Kadowaki T, Kasuga M, Akanuma Y. Decreased autophosphorylation of the insulin receptor kinase in STZ diabetic rats. *J. Biol. Chem* 1984; 259: 14208-216.
10. Patricia Ordonez. Insulin sensitivity in STZ induced diabetic rats treated with different doses of 17 β -estradiol or progesterone. *Exp Physiol* 2006, 92(1): 241
11. Henriksen EJ. Oxidative stress and antioxidant treatment effect on muscle glucose transport in animal models of type-1 and type-2 diabetes. *Anti-oxidants in diabetes management*. 1st edition, pg 303-17, NY.
12. Brozowski T, Konturek SJ et al. Role of L-arginine, a substrate for nitric oxide synthase in gastro-protection and ulcer healing. *J Gastroenterol* 1997; 32(4): 442-52.
13. Willmot M, Gray L, Gibson C, Murphy S, Bath PM. A systematic review of nitric oxide donors and L-arginine in experimental stroke; effects on infarct size and cerebral blood flow. *Nitric Oxide* 2005; 12(3): 141-9.
14. E.M. Repetto, R. Sanchez, J. Gipelli, F. Astort, C. Dysregulation of corticosterone secretion in STZ diabetic rats. Modulatory role of adrenocortical nitrenergic system. *Endocrinology* 2010; 151(1): 203-10.
15. Naqvi A (1992). The effects of STZ on duodenal mucosa in albino rats. M.Phil thesis, Basic Medical Sciences Institute, JPMC, Karachi.
16. Quddus I (2010) Effect of fat-induced stress on adrenal gland with protective role of L-arginine in albino rats. M.Phil thesis, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi.
17. Bancroft JD and Cook HC (1994) Manual histological techniques and their diagnostic application. Edinburgh: Churchill Livingstone, pp 63-64, 135-36.
18. Thomas J. Rosol, John T. Yarrington, John Latendresse. *Toxicol Pathol* 2001; 29(1): 41-48.
19. Yarrington JT, Loudy DE, Sprinkle DJ, Gibson JP, Wright CI, Johnston JO. Degeneration of the rat and canine adrenal cortex caused by alpha – (1,4,dioxide-3-methyl quinoxalin-2-yl)-N-methylnitron(DMNM). *Fund. Appl. Toxicol* 5: 370-81.
20. S. McGinn, P. Porunnik, M. King. High glucose and endothelial cell growth: novel effects independent of autocrine TGF-B1 and hyperosmolarity. *Am J Physiol Cell Physiol* 2003;284 (6): C1374-86.
21. Astort F, Repetto EM, Martinez Calejman C, Cipelli JM, Sanchez R, Dr Gruccio JM, Mercau M. High glucose-induced changes in steroid production in adrenal cells. *Diabetes Metab Res Rev* 2009; 25: 477-486.
22. Owen Chan, Karen Inouy, Eitan Akirav, Edward Park. Insulin alone increases hypothalamo pituitary adrenal activity and diabetes lowers peak stress responses. *Endocrinology* 2005; 146(3): 1382.
23. Cameron LA, Hinson JP. Role of nitric oxide derived from L-arginine in the control of steroido-genesis and perfusion medium flow rate in the isolated perfused rat adrenal gland. *J Endocrinol* 1993;139: 415 -23.
24. Rebuffat P, Belloni AS, Malendowicz LK, Mazzocchi, Meheghelliv, Nussdorfer GG. Effects of STZ induced experimental diabetes on the morphology and function of the zona fasciculata of rat adrenal cortex. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1988; 56: 13-19.
25. Evans JL, Maddux BA, Goldfine ID. The molecular basis for oxidative stress induced insulin resistance. *Antioxid Redox Signal* 2005; 7: 1040-52.
26. Pier Marco Piatti, Lucilla L. Monti, Gianpietro Valsecchi. Long term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type-2 diabetic pts. *Diabetes Care* 2001;24(5): 875-80.
27. Craig J. Hanke, Timothy O'Brien. Campbell. Inhibition of adrenal cell aldosterone synthesis by endogenous NO release. *Hypertension* 2000; 35: 324-28.
28. Zafar M, Naqvi SNH (2010) Effect of STZ-induced diabetes on the relative weight of kidney, liver and pancreas in albino rats; a comparative study. *Int. J Morphol.*, 28(1): 135-42.
29. Koholi R, Meininger CJ, Hayner ET, Yan W. Dietary L-arginine supplementation enhances endothelial nitric oxide synthesis in streptozotocin-induced diabetic rats. *J. Nutrition*, 2004(134); 3: 600-6.

