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Morphological and Functional Assessment of Chronic Dexamethasone-Induced Adrenal Atrophy in Mice

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Abstract: ***Background:*** *Prolonged administration of synthetic glucocorticoids, such as dexamethasone (DEX), exerts profound negative feedback on the hypothalamic-pituitary-adrenal (HPA) axis, leading to iatrogenic adrenal insufficiency. While the systemic immunosuppressive effects of DEX are well-documented, the precise correlation between structural adrenocortical involution and the functional loss of stress reactivity requires deeper elucidation.*

Objective: *This study assesses the morphological alterations and functional deficits in the adrenal glands of mice following chronic dexamethasone exposure.*

Methods: *Adult male Parkes strain mice were divided into Control and DEX-treated groups. The experimental group received DEX (400 µg/kg/day, i.p.) for 30 consecutive days. We evaluated body weight trajectories, adrenal histomorphometry (cortex width and zona fasciculata cell size), and basal plasma corticosterone levels. Furthermore, an acute lipopolysaccharide (LPS) challenge was utilized to assess the secretory reactivity of the suppressed HPA axis.*

Results: *Chronic DEX administration resulted in significant growth retardation and profound structural atrophy of the adrenal cortex, characterized by a marked reduction in mean cortical width and zona fasciculata cellular shrinkage. Functionally, DEX-treated mice exhibited severe suppression of basal corticosterone and a completely blunted secretory response to acute LPS immune challenge compared to controls.*

Conclusion: *Chronic dexamethasone treatment induces parallel morphological regression and functional exhaustion of the adrenal cortex. The blunted response to LPS highlights the severe risk of adrenal crisis during physiological stress following prolonged corticosteroid therapy.*

Keywords: Glucocorticoids (GCs), Dexamethasone, Adrenal, Corticosterone, Atrophy, LPS, Zona fasciculata, HPA axis, Mice.

I. INTRODUCTION

Synthetic glucocorticoids, prominently dexamethasone (DEX), are cornerstone therapeutic agents in modern medicine due to their unparalleled anti-inflammatory, antineoplastic properties and immunosuppressive efficacy (Coutinho & Chapman, 2011). Due to their profound anti-inflammatory, immunosuppressive, and antineoplastic properties, they are routinely utilized in the management of autoimmune disorders, severe asthma, rheumatoid arthritis, and acute exacerbations of chronic inflammatory diseases and post-transplant management. However, the therapeutic efficacy of chronic glucocorticoid administration is inextricably linked to severe iatrogenic toxicities. The most critical and potentially life-threatening of these adverse effects is the profound, sustained suppression of the endogenous hypothalamic-pituitary-adrenal (HPA) axis, leading to secondary adrenal insufficiency (Chrousos, 1995, Nicolaides et al., 2015).

Under homeostatic conditions, the HPA axis operates via a tightly regulated circadian rhythm and is highly responsive to physiological and psychological stressors. The hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). ACTH then binds to the melanocortin-2 receptor (MC2R) on the cells of the adrenal cortex, stimulating the synthesis and secretion of endogenous glucocorticoids (cortisol in humans, corticosterone in rodents) (Smith & Vale, 2006).

Exogenous administration of highly potent, long-acting synthetic GCs like dexamethasone bypasses this physiological regulation. DEX exerts continuous, powerful negative feedback on both the paraventricular nucleus (PVN) of the hypothalamus and the corticotropes of the anterior pituitary. This effectively halts the secretion of CRH and ACTH (Sapolsky et al., 2000). Because ACTH functions not only as a secretagogue but as the primary trophic (growth-promoting and survival) factor for the adrenal cortex, its chronic absence leads to rapid and severe tissue involution.

The structural involution induced by ACTH deprivation is predominantly localized to the zona fasciculata—the thickest layer of the adrenal cortex responsible for glucocorticoid synthesis. Morphologically, this manifests as a significant reduction in overall cortical width and dramatic cellular hypotrophy (compacted cells with reduced cytoplasmic-to-nuclear ratios and depleted lipid droplets) (Ulrich-Lai et al., 2006).

At the molecular level, the lack of ACTH signaling downregulates the expression of critical steroidogenic machinery. This includes the steroidogenic acute regulatory protein (StAR), which is responsible for the rate-limiting transport of cholesterol into the mitochondria, as well as key cytochrome P450 enzymes such as cholesterol side-chain cleavage enzyme (CYP11A1) and 11β-hydroxylase (CYP11B1) (Gallo-Payet, 2016). Consequently, the adrenal gland is not merely "resting" during chronic DEX therapy; its physical and biochemical infrastructure is actively dismantled.

The clinical danger of this structural atrophy becomes apparent during episodes of acute systemic stress. A healthy HPA axis relies on robust immune-neuroendocrine crosstalk. During a bacterial infection, for example, pathogen-associated molecular patterns like lipopolysaccharide (LPS) trigger immune cells to release a storm of pro-inflammatory cytokines, notably Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α). These cytokines powerfully stimulate the HPA axis to release massive amounts of corticosterone, which in turn acts as a vital physiological brake to prevent the immune system from spiralling into fatal systemic inflammatory shock.

In a state of chronic dexamethasone-induced atrophy, this life-saving reflex is broken. While basal corticosterone levels may be chronically low, the true functional deficit is revealed when the system is challenged. The severely atrophied zona fasciculata lacks the structural capacity to rapidly synthesize and secrete corticosterone in response to stress signals, leaving the organism highly vulnerable to adrenal crisis and hemodynamic collapse.

While the biochemical suppression of the HPA axis following glucocorticoid therapy is widely acknowledged, comprehensive in vivo models correlating the precise degree of cellular morphometric shrinkage with the functional loss of stress reactivity remain limited. The present study utilizes a 30-day murine model of chronic dexamethasone administration to provide a definitive mapping of this dual pathology.

By combining detailed morphometric analysis of the zona fasciculata with a functional assessment using an acute LPS immune challenge, this study aims to clarify the timeline and severity of iatrogenic adrenal exhaustion. We hypothesize that chronic DEX exposure will induce profound cellular hypotrophy in the adrenal cortex, directly resulting in a blunted secretory response to LPS-induced cytokine signalling, thereby highlighting relationship between structural cortical degradation and the functional failure of the adrenal gland to respond to immune-mediated stress.

II. MATERIALS AND METHODS

ANIMALS

Albino mice (Parkes Strain) were procured from Central Drug Research Institute Lucknow, India and housed in PVC cages (290x320x390 mm) under standard laboratory conditions in 12:12 h light/dark cycle (lights on between 07:00 and 19:00) in a temperature (21 ± 2 ºC) and humidity (55 ± 5%) controlled room. All the animals were having free access to water and mice feed (pelleted mice feed) ad libitum. They were acclimatized to the laboratory condition for one week before experimentation. The care and handling of the animals were according to the ethical guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India.

EXPERIMENTAL DESIGN

Thirty adult male mice (30±2 g BW) were divided into two groups of fifteen each.

Group I mice were given intraperitoneal injection of dexamethasone 21-phosphate (400 µg/kg BW/day, i.p) for thirty consecutive days in sterile pyrogen free saline (DEX-group).

Group II mice received an equal volume of sterile pyrogen free saline for the same period and are treated as control group (CONT-group).

All the injections were given at evening hours between 4:15pm to 4:45pm. i.e. coincident with the circadian increase in endogenous pineal and HPA secretory activity for 30 consecutive days. Mice of both the groups were weighed on every second day till the end of the experiment. On the next day of last treatment, ten animals from each group of both the experiments were sacrificed to collect desired tissues (adrenal and pituitary) and blood sample. Remaining five animals (of each group) were used for LPS challenge test.

III. LIPOPOLYSACCHARIDE (LPS) CHALLENGE TEST

Lipopolysaccharide (LPS) is an endotoxin. It is the principal component of the external wall of Gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, and Salmonella typhimurium. It is considered to be a systemic stressor eliciting a prolonged activation of the hypothalamic-pituitary-adrenal axis. The HPA axis response after challenge to this endotoxin releases cytokines (IL-1, IL-6 and TNF-a) from stimulated peripheral immune cells which in turn stimulate HPA axis at different levels resulting in elevated plasma corticosterone level. Thus, it is also termed as immunological stressor. In the present study, LPS was used to assess the secretory response/reactivity of HPA axis in different experimental groups. At the end of both the experiments, remaining animals from each group were immune challenged with LPS and blood samples were collected after two and three hours to assess the response of pituitary-adrenal axis to acute LPS stress.

IV. TISSUE PREPARATION

On completion of the experiments, cardiac blood was sucked from ether anaesthetized mice using heparinized micro-syringe and collected in microcentrifuge tubes for further analysis. After blood sampling, the mice were decapitated, adrenal glands were immediately dissected out, freed from the adjacent tissues, blotted and weighed. The adrenal tissues were then fixed in Bouin’s fluid for further histological study. After overnight fixation the tissues were washed thoroughly and processed conventionally for paraffin embedding.

V. ROUTINE HISTOLOGICAL STAINING AND MORPHOMETRY

Paraffin blocks of and adrenal glands of all mice were cut at 5-7 micron thickness and stretched on glass slides. After deparaffinization, sections were processed for hematoxylen-eosine staining to be observed under light microscope. Sections were randomly selected for the study of morphological changes. Histopathological changes were photographed using Nikon Photomicroscope. The size of zona fasciculata cells of adrenal cortex were determined for 50 cells randomly selected from sections of each group. For zona fasciculata cells both axes were measured and average was taken as size. Adrenal cortex width was measured at several sites and average was taken to determine the mean cortex width. All the measurements were done using the Image J 1.32 image analysis software package (NIH, USA).

VI. CORTICOSTERONE ASSAY

Blood samples were centrifuged at 3000 r.p.m. for 10 min. The plasma supernatant was collected and stored at -20 °C until hormonal assay. The assays were carried out as per the protocol of the manufacturer of the ELISA Kit. Samples from each replicate batch of control as well as experimental groups were pooled and assayed in duplicates. Before proceeding with the assay, all the reagents, serum references and samples were brought to room temperature (20–27 °C). Corticosterone Assay Corticosterone was assayed using commercially available Corticosterone ELISA Kit (NEOGEN Corporation, USA). The results are expressed as Mean ± S.E ($n \geqslant 5$).

VII. STATISTICAL ANALYSIS

The results are expressed as Mean ± S.E ($n \geqslant 5$). Statistically significant differences between the treatment groups were analyzed using one-way analysis of variance (ANOVA) followed by post hoc comparison using the Tukeys test. The differences of the means were considered significant when $p \leqslant 0.05$. For all statistical analysis, the Statistical Package of Social Science version 10.0 (SPSS Inc.) was used.

VIII. RESULTS

Effect on Body Weight and General Morphology

Chronic administration of dexamethasone for 30 days significantly impacted the overall growth trajectory of the mice. While the control group (CONT) demonstrated a steady increase in body weight throughout the experimental period, the DEX-treated group exhibited marked weight stagnation and eventual weight loss by day 30 ($p < 0.05$), data not shown.

Effect on Adrenal Weight

Chronic DEX treatment significantly decreased ($P \leqslant 0.01$) the absolute as well as relative adrenal weights (Fig. 1).

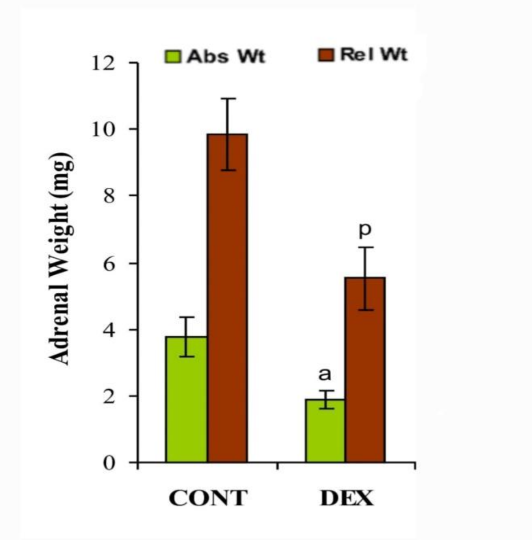

Figure 1 Graph showing absolute (Abs) and relative (Rel) adrenal weights under dexamethasone (DEX) and Control (CONT) treatment. Data represented as Mean ± SEM ($n \geqslant 5$). (a & p) $p < 0.001$ DEX vs CONT.

Effect on Histomorphometry of the Adrenal Cortex

Histological examination of H&E-stained adrenal sections revealed profound histopathological alterations in the DEX-treated mice. The mean width of the adrenal cortex was significantly reduced in the DEX group compared to the CONT group ($p < 0.01$). The three distinct cortical zonations, zona glomarulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) as observed in CONT became indistinct in DEX group (Figs. 2). The arrangement pattern of fasciculata cells of the CONT in strand (Fig. 3b) was disrupted in DEX group (Fig. 3d). The number of cortical cells was less together with a significant decrease in density and size ($P \leqslant 0.01$) of ZF cells as compared to CONT group (Figs. 3c). Morphometric analysis utilizing ImageJ software demonstrated severe cellular atrophy. The mean cross-sectional area of zona fasciculata cells in DEX-treated mice was drastically diminished, appearing compacted with dense nuclei and reduced cytoplasmic volume compared to the robust, lipid-rich cells observed in the controls ($p < 0.01$).

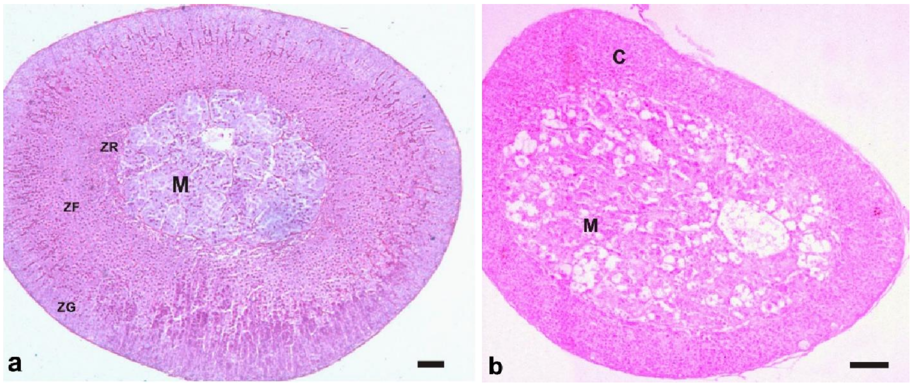

Figure 2 Cross sections showing adrenal histology of control (CONT, a) and dexamethasone (DEX, b) treated groups. Note the disruption of cortex (C) and medulla (M), reduced cortical width and loss of zonation in DEX group (b) compared to CONT (a). Hematoxylin and Eosin. Bar 50 m.□

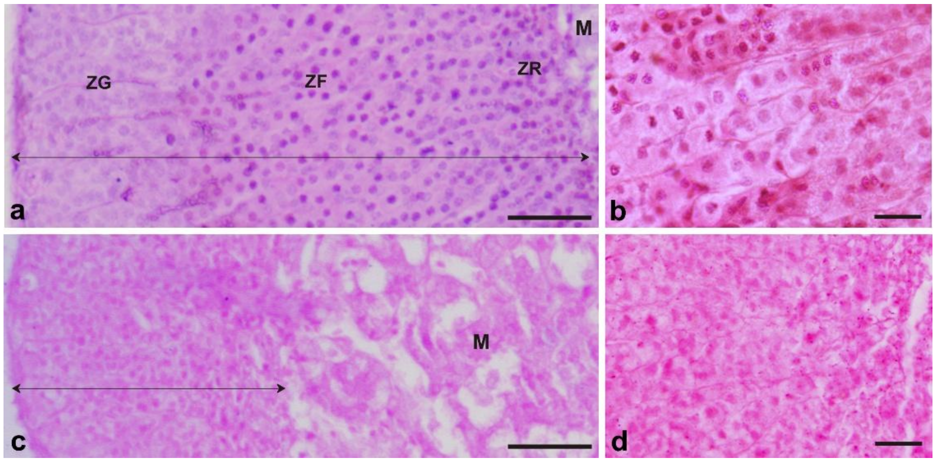

Figure 3 Higher magnification of adrenal showing distinct cortical zonation (a) and arrangement of fasciculata cells in cord (b) in control. Note reduction of cortical width and loss of cortical zonation (c) and disrupted arrangement of fasciculata cells (d) in DEX group. Hematoxylin and m (b, d).□m (a, c); 20 □Eosin. Bar 50

Effect on histomorphometry of the Adrenal Medulla

The adrenal medulla was also significantly altered under chronic DEX treatment. The normal oval shape and organization of chromaffin cells in follicles as observed in CONT (Fig. 4a) was totally disrupted in DEX group (Fig. 4b).

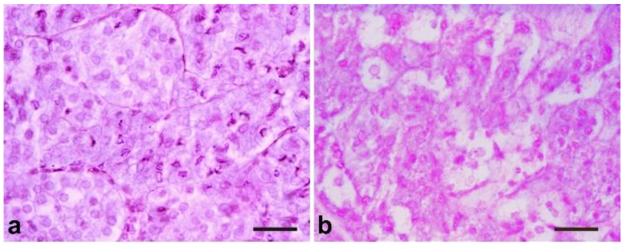

Figure 4 Higher magnification of adrenal medulla showing chromafin cells histology. Note disruption of chromafin cells m.□in DEX group (b). Hematoxylin and Eosin. Bar 20

Basal Plasma Corticosterone Levels

Functional suppression of the adrenal gland was confirmed via ELISA (Fig. 5). At the end of the 30-day treatment period (prior to the LPS challenge), A significant decrease in the basal plasma level of corticosterone was recorded in DEX group as compared to vehicle treated CONT group ($p < 0.01$).

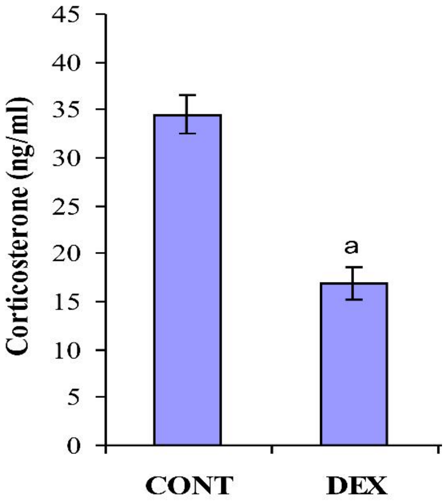

Figure 5 Graph showing plasma corticosterone level under dexamethasone (DEX) and vehicle (CONT) treatment. Data represented as Mean ± SEM ($n \geqslant 5$). a, $p < 0.01$ DEX vs CONT.

HPA Axis Reactivity to Acute LPS Challenge

To assess the secretory reserve and stress reactivity of the HPA axis, an acute LPS immune challenge was administered. Secretory response of HPA axis to acute LPS stress was drastically affected on DEX treatment. Two hours after acute LPS exposure, the plasma CORT level was significantly increased in both treatment groups (Table 5). The elevation of CORT was highest in CONT (9 folds approx.) and least in mice treated with DEX (5 folds approx). Four hours after LPS stress a significant decrease in plasma CORT level (similar to prestress basal value) was observed CONT group but DEX group still showed higher CORT level than the basal value, indicating a completely blunted secretory response and severe functional adrenal insufficiency (Table 5).

Groups	Plasma Corticosterone (ng/ml)		
	Basal	**Two hrs after LPS Challenge**	**Four hrs after LPS Challenge**
CONT	34.01± 4.01	315.05 ± 15.75	36.75 ± 3.02
DEX	16.91 ± 1.78 a	88.95 ± 18.95 b	81.23 ± 10.2 c

Table 5 Plasma corticosterone of different treatment groups subjected to LPS. Data are represented as Mean ± SEM (n = 5). (a, b & c) $p < 0.01$ DEX vs CONT.

Discussion

The present investigation provides a definitive correlation between the structural involution of the adrenal cortex and the functional exhaustion of the HPA axis following chronic dexamethasone administration. Our findings confirm that 30 days of DEX exposure induces severe morphological regression of the zona fasciculata, which directly translates into adrenal insufficiency resulting its inability to mount an endogenous stress response during an acute immune challenge. The adrenal involution was prominent as revealed by significant weight reduction and disrupted histopathology of both cortex as well as medulla. Significant reduction of plasma CORT level, the hormonal product of this axis, was also noted.

These results are in line with various studies reporting the hypofunctioning/dysregulation of HPA axis by high GC level either stress-induced (Smith et al., 1991; McEwen, 1992; Landfield and Eldridge, 1994; Aguilera, 1994) or due to long-term synthetic GC treatment (De Kloet et al., 1974; Young et al., 1995; van Haarst et al., 1996; Buwalda et al., 1999). The inhibition of the HPA axis resulted due to the negative feedback of the glucocorticoid at the level of pituitary and different sites in the brain as demonstrated by many investigators (Munck and Guyre, 1984; Keller-Wood and Dallman, 1984; Dallman et al., 1987, 2000; De Kloet, 1991; Akana et al., 1992; Shipston, 1995; Watts, 2000; Tsigos and Chrousos, 2002).

The profound reduction in mean cortical width and zona fasciculata cell size observed in the DEX-treated mice is a direct consequence of prolonged ACTH deprivation. ACTH regulates not only the expression of key steroidogenic enzymes (such as StAR and CYP11A1) but also maintains cellular hypertrophy and vascularization within the adrenal cortex (Gallo-Payet, 2016). The continuous negative feedback exerted by high-dose exogenous DEX effectively starved the adrenal glands of this trophic signaling, leading to cellular compaction and cytoplasmic volume loss. This aligns with previous histological studies demonstrating that synthetic glucocorticoids induce rapid hypotrophy in steroidogenic tissues (Ulrich-Lai et al., 2006).

Functionally, this structural degradation manifested as severe secondary adrenal insufficiency. The suppression of basal corticosterone levels in the DEX group was expected; however, the LPS challenge test provided critical insight into the gland's functional reserve. Lipopolysaccharide, a bacterial endotoxin, is a potent activator of the immune system, normally triggering the release of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) that strongly stimulate the HPA axis to release corticosterone, thereby preventing systemic inflammatory shock (Turnbull & Rivier, 1999).

In our study, the control mice exhibited the anticipated robust corticosterone spike at 2 hours post-LPS. In stark contrast, the DEX-treated mice exhibited a "flatline" less response. This blunted reactivity indicates that the zona fasciculata cells were not merely resting, but were functionally incapacitated and structurally incapable of rapid steroidogenesis even when presented with a massive physiological stressor. By administering the DEX injections in the late afternoon (16:15–16:45), our protocol coincided with the natural diurnal rise in endogenous corticosterone, likely maximizing the suppression of the circadian HPA pacemaker (Kalsbeek et al., 2012).

Clinically, these data underscore the precise histopathological reality behind "steroid withdrawal syndrome." The morphological atrophy is so severe that simply removing the synthetic glucocorticoid is insufficient; the zona fasciculata requires significant time to physically rebuild its cellular machinery before it can safely manage homeostatic stress.

IX. CONCLUSION

Chronic dexamethasone treatment in mice induces a dual pathology of the adrenal gland: structural hypotrophy of the zona fasciculata and complete functional unresponsiveness to immune-mediated stress. These findings emphasize the necessity of strict tapering protocols in clinical settings to allow for the gradual regeneration of adrenocortical tissue and the restoration of life-saving HPA axis reactivity.

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