

Histological Changes in the Gingival Tissues of Rats Subjected to Surgical Extraction of the Adrenal Glands

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Abstract: Hormones are specific regulatory molecules that have potent effects on the major determinants of the development and the integrity of the skeleton and oral cavity also including periodontal tissues. The aim of this study, is to investigate the effect of adrenalectomy on rat gingival tissue. Twenty-eight mature *Wistar albino* female and male rats divided randomly into 2 groups and in one group adrenalectomy was performed. After 21 days recovery period, Control and Experimental groups were sacrificed under Ketamin HCl anesthesia and clinical and histological observations were made. Microscopic findings, revealed significant changes in adrenalectomized rats. Hypertrophy and thickness of gingival epithelium was apparent in the gingival epithelium of ADX group. Increase in mitotic activity in stratum basale layer of gingival epithelium and increase in keratinisation layer were observed in adrenalectomized rats. A decrease in collagen fibrils and irregularity of papilla was seen in adrenalectomized rats. Our findings demonstrate the importance of adrenal glands in the development of gingival tissue and suggest that adrenal glands deficiency is one mechanism, by which gingival disorder susceptibility may be increased.

Key words: Gingival tissue, adrenalectomy, rat, histological changes, weight of liver, kidney and hypophysis

INTRODUCTION

Hormones are specific regulatory molecules that modulate reproduction, growth and development, maintenance of the internal environment, as well as energy production, utilization and storage (Mariotti, 1994). Hormonal effects reflect physiological/pathological changes in almost all types of tissues of the body (Gornstein *et al.*, 1999).

The adrenal glands are paired endocrine glands, one located above each kidney. The adrenal gland is important in producing steroid, both corticosteroid and mineral corticoids. If the adrenal gland does not produce these hormones, the loss leads to devastating effects in nearly every organ system and eventually death (McGivern and Redei, 1994). Adrenal insufficiency may cause persistent vomiting, anorexia, hypoglycemia, poor weight gain in a child or unexplained weight loss in an adult, malaise, fatigue, muscular weakness, unexplained isotonic or hyponatremic dehydration, hyperkalemia, hypotension, hypoglycemia (Kendereski *et al.*, 1999). One of the most definitive signs of primary adrenal

insufficiency is widespread hyperpigmentation of the skin and buccal mucosa, leading to a tanned appearance most prominent on the face, neck, elbows, knees and palmar creases (Luken, 1999). The object of this study, was to investigate histological changes in the gingival tissues of rats subjected to surgical extraction of the adrenal glands.

MATERIALS AND METHODS

Twenty-eight mature *Wistar albino* female and male rats were obtained from University of Dicle, Medical Science and Application Center (DUSAM). All animals received humane care according to criteria of the National Academy of Sciences.

The experiments were approved by the Committee on Ethics of University of Dicle. The ambient temperature (22°C) and relative humidity (45%) were maintained throughout the experiments. The rats were divided randomly into 2 groups: the animals served as control group (n: 10) (n: 18) Under Ketamine HCl (50 mg kg⁻¹) and Xylazine (10 mg kg⁻¹) anaesthesia the bilateral adrenal glands were removed (ADX). The rats were given

20 g day⁻¹ saline and 1.1% Ca drinking water *ad libitum* after surgery to prevent dehydration-Addison crisis. After giving 21 days of recovery period to the rat, control and experimental groups were sacrificed by Ketamin HCl.

Gingiva of molar region was removed and tissues were fixated with 5% neutral formalin solution (pH: 7.4). Tissue pieces were dehydrated through graded ethanol series. Maxilla of molars region were embedded in paraffin. Paraffin blocks were cut and stained with Hematoxylin-Van Giesson (Edna *et al.*, 1992).

The liver, kidney and hypophysis weights were noted. Statistical validation was accomplished using the Mann-Whitney U-test (Goerge and William, 1973).

RESULTS AND DISCUSSION

Clinical appearance and weight of organs: In the control group the gingiva had a healthy macroscopic appearance, with a bright colour ranging from pink to red. In those rats subjected to Adrenalectomy (ADX), mild oedema and a duller colour were observed in the gingiva. Statistical comparison revealed no significant difference in kidney, pituitary gland and liver weights between the male rats in the control and ADX groups ($p > 0.05$) ($p > 0.05$, Table 1 and 2).

However, a significant decrease was observed in liver weights in female rats subjected to ADX ($p = 0.003$).

Histological findings

Control groups: We observed a normal, flat, multi-layered, keratinized epithelial appearance in the gingival epithelium. In the gingival lamina propria the collagen fibres had a regular appearance and microscopic papillae of the epithelium were also normal.

In the gingival epithelia of both male and female rats in the control group, we observed a normal keratinization layer and keratocytes (Fig. 1a, b).

Experimental groups: We observed an increase in epithelial thickness, hyperplasia and hypertrophy in epithelial cells in the gingiva of rats subjected to surgical extraction of the adrenal glands. There was mitotic activity in the stratum basale layers of the gingival epithelia, together with thickening of the epithelium corneum layer. There were a striking increase in microscopic papillae depth, together with a decrease and irregular structure in the collagen fibres in the lamina propria. One of our significant findings was an increase in keratinization in the epithelia (Fig. 1c, d).

As with other tissues, hormones affect the periodontium. The aim of this study was to determine what histological changes might arise in the gingival tissues of rats subjected to surgical extraction of the adrenal glands.

The most important histological finding in our study was an increase in epithelial thickness, hyperplasia and a hypertrophic appearance in the epithelial cells and a thickening of the epithelial corneum layer. In addition, there was a noticeable decrease and irregularity in the collagen fibres in the lamina propria.

Kilinc *et al.* (2005) reported that adrenalectomy had a negative effect on skin layers. In our study, we determined that adrenalectomy had a negative effect on the gingiva.

Trejo *et al.* (1995) reported that glucocorticoid hormones play a role in the division and maturation of

Table 1: Weights of Organs of adrenalectomized and control female rats (Mean±SD)

Organ's weight (g)	Groups	N	Mean	SD	Mean rank	Mann-whitney	
						U-value	p-value
Liver	Control	5	6.4340	0.4023	3.00	0	0.003
	ADX	8	9.0587	1.1559	9.50		
Right kidney	Control	5	1.1720	0.2125	8.00	15	0.464 ns
	ADX	8	1.1038	0.2337	6.38		
Left kidney	Control	5	1.0380	0.2192	6.20	16	0.558 ns
	ADX	8	1.1088	0.2380	7.50		
Hypophysis	Control	5	0.0404	0.0018	5.40	12	0.242
	ADX	8	0.0308	0.0551	8.00		

Table 2: Weights of organs of adrenalectomized and control male rats (Mean±SD)

Organ's weight (g)	Groups	N	Mean	SD	Mean rank	Mann-whitney	
						U-value	p-value
Liver	Control	5	9.7960	1.0008	5.60	13	0.142 ns
	ADX	10	11.1560	2.7575	9.20		
Right kidney	Control	5	1.5440	0.1417	5.40	12	0.111 ns
	ADX	10	1.7100	0.3524	9.30		
Left kidney	Control	5	1.3900	0.1231	5.80	14	0.178 ns
	ADX	10	1.5350	0.3064	9.10		
Hypophysis	Control	5	0.0119	0.0014	8.00	25	1.000 ns
	ADX	10	0.0116	0.0016	8.00		

ns: non significant

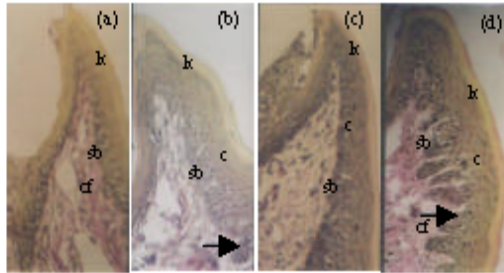


Fig. 1: Histological appearance of gingival epithelium of control female. Histological appearance of gingival epithelium of Adrenalectomized (ADX) female rat. Histological appearance of gingival epithelium of control male rat. Histological appearance of gingival epithelium of Adrenalectomized (ADX) male rat; k: keratinisation, sb: stratum basale, arrow: stratum papillare, c: keratinocyte, cf: collagen fibrils (Hematoxyline-V an Giesson, X40)

cerebral cortex cells in prenatal rats. We determined mitotic activity in the stratum basale layer of the gingival epithelium and a thickening of the epithelial corneum layer. We are unable to discuss histological studies in this area as these are limited in the literature.

Some researchers have reported that one of the most significant indicators of adrenal hormone insufficiency is hyperpigmentation in the dermal and oral mucosa (Kendereski *et al.*, 1999; Luken, 1999). But since, this is a known indicator, we did not investigate melanocytes or hyperpigmentation.

At the same time, we considered the weights of various rat organs and one major finding was a statistically significant decrease in liver weight in female rats subjected to adrenalectomy compared to the control group. The best-known metabolic effects are their impact on the liver, accelerating the formation of glucose from proteins and fats (gluconeogenesis) and their raising blood glucose (sugar) levels (Greenspan and Gardner, 2001). We therefore, think that the liver will be more affected than other organs by a deficiency in these hormones.

CONCLUSION

This study shows that adrenalectomy leads to an increase in gingival epithelium thickness, an increase in

microscopic papillae depth and a decrease and irregularity in collagen fibres in the lamina propria.

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