The effects of sialoadenectomy & flutamide on skin development

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Summary

Background: Epidermal growth factor is a low molecular weight polypeptide with 53 amino acids and is known to stimulate cell proliferation and differentiation in a wide range of tissues. The submandibulary gland in the mouse is a rich source of epidermal growth factor and decreased plasma epidermal growth factor levels have been observed after sialoadenectomy (removal of the submandibular glands). Furthermore, there is evidence that epidermal growth factor stimulates spermatogenesis and reverses antiandrogen induced cryptorchidism.

Objective: In the present study, the teratogenic effects of sialoadenectomy and antiandrogen (flutamide) administration on rat skin were investigated histologically.

Materials & Methods: Thirty Spraque-Dawley female rats were separated into three groups (n=10), a control (sham-operated) and two experimental groups. The first experimental group of rats were subjected to sialoadenectomy in order to create maternal EGF deficiency one month before copulation. The second experimental group of rats were given flutamide (10 mg/100 g) for ten days during pregnancy. Three months after birth, a penile skin biopsy was taken from respective offspring in all groups.

Results: A statistically significant reduced body weight and length were noted in the first group of litters (maternal EGF deficient) and in the flutamide administered group when compared to the control group. Atrophic epidermis and dermal adnexa were observed histologically as the teratogenic effects of sialoadenectomy and flutamide administration on rat skin development.

Conclusion: Epidermal growth factor is a key hormone for skin development and antiandrogen administration may insult this process by interfering with epidermal growth factor metabolism.

Key words: Epidermal growth factor; Flutamide; Sialoadenectomy; Skin; Teratology.

Introduction

Epidermal growth factor (EGF) is a mitogenic polypeptide hormone which in vivo stimulates ectodermal (epithelial) and endodermal cell growth and in vitro growth of epithelial cells and fibroblasts [1]. However, transcripts of EGF have not been found in epidermis. EGF is formed in the salivary glands, kidney tubules and intestinal tract and occurs in nanogram quantities in plasma. Administration of androgens, progestins and adrenergic agents have been shown to increase the production of EGF in the submandibular gland thus creating a rich source of EGF in the circulation. It has also been demonstrated that sialoadenectomy of male mice led to a marked decrease in the sperm content of the epididymis [2]. Flutamide is a nonsteroidal compound with some antiandrogenic properties which appears to act by inhibiting the uptake and/or binding of androgens in target tissues. It has been used in combination with gonadrelin analogues for the treatment of prostatic carcinoma. In dermatological practice, flutamide has also been used in the treatment of alopecia and hirsuitism in females.

The purpose of the present study was to investigate histologically the teratogenic effects of sialoadenectomy and flutamide administration on rat skin development.

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Materials and Methods

Thirty Spraque-Dawley female adult rats (obtained from the Department of Medical Science Application and Research Centre of Dicle University) were separated into a control and two experimental groups (n=10). All rats were fed standard pellet food during the study.

The first experimental group of rats were subjected to removal of the submandibular glands under general anesthesia, whereas in the control group of rats only a transverse neck incision was performed under general anesthesia (sham-operated).

The second group of experimental rats were not subjected to sialoadenectomy. One month later, all female rats were confined in a special cage over 48 hours for copulation with adult males.

The second group of experimental gravid rats were administered flutamide orally in a dosage of 10 mg/100g/day between 10-20 days of pregnancy. During pregnancy, all experimental gravids were examined daily using real time ultrasonography (Toshiba SSA-270A, 7.5 MHz linear transducer) to count the number of fetuses and detect the fetal cardiac activation.

The gravid rats gave birth during the 21st and 22nd days of gestation. After birth, female litters were excluded and male litters were weighed and their lengths were measured. Three months later, the male rats underwent a penile skin biopsy. The tissues were fixed in 10% formaldehyde and then embedded in paraffin wax, serial sectioned and stained with hematoxylin-eosin for evaluation using a light microscope.

Results

During the first week of gestation, the fetuses could not be detected with ultrasonographical examination because of their small size. There were no resorbed fetuses or stillborns after the first week of gestation. The average number of male and female litters was the same in the control and exposed dams (Tables 1, 2). The mean weight and length of the first group of litters (maternal EGF deficient) and the flutamide administered group of litters were less than the control group of litters (Table 3). In statistical analysis, the weights were analyzed by ANOVA procedures followed by a multiple comparison procedure based on the Tukey-HSD method. The results of the ANOVA were significant ($p<0.0001$) and the multiple comparison procedure indicated that the following treatments were different at $p<0.05$:

– Control group *versus* 1st experimental group;
– Control group *versus* 2nd experimental group;
– 1st experimental group *versus* 2nd experimental group.

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– Control group *versus* 1st experimental group;
– Control group *versus* 2nd experimental group.

At histopathological examination, normal epidermis and dermis together with dermal adnexa were observed in the control group of rats (Figures 1, 2). In the first experimental group of rats, hyperkeratosis and/or parakeratosis were noted on atrophic epidermis. Atrophic sebaceous glands and decreased number of sebaceous glands together with hair follicles were also noted in the first experimental group (Figures 3, 4). Similar findings were more prominent in the flutamide administered group of rats. Furthermore, loss of rete ridges were observed in focal areas of the atrophic epidermis in this group (Figures 5, 6). A summary of the histopathological findings is presented in Table 4.

Discussion

The submandibulary salivary gland appears to secrete two important hormonal growth factors: the epidermal growth factor (EGF) and the nerve growth factor (NGF) [3]. EGF plays a role in a variety of biological actions including promotion of epidermal development, wound healing, eruption of the incisors, activation of various transport systems and changes in cellular metabolism in addition to mitogenesis, stimulation of pituitary secretion of ACTH and GH, and inhibition of gastric and of thyroid hormone secretion. Moreover, most evidence indicates that it is an important hormone in the male reproductive system. EGF receptors have been demonstrated in the

Fig. 1

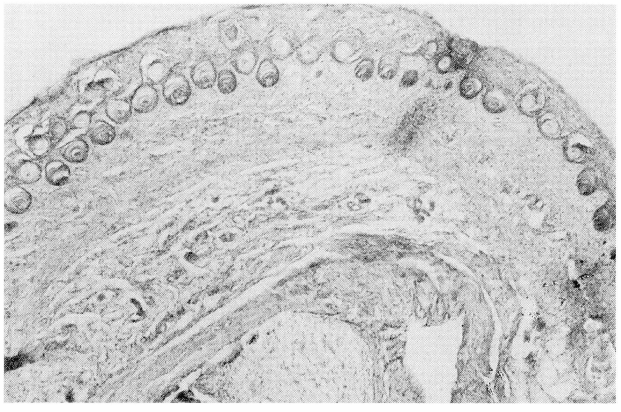


Fig. 2

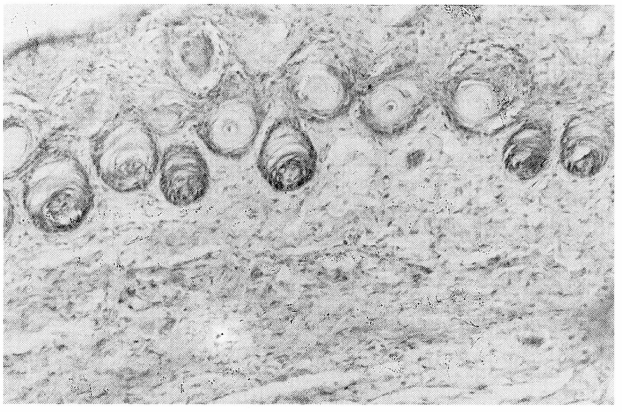


Fig. 3

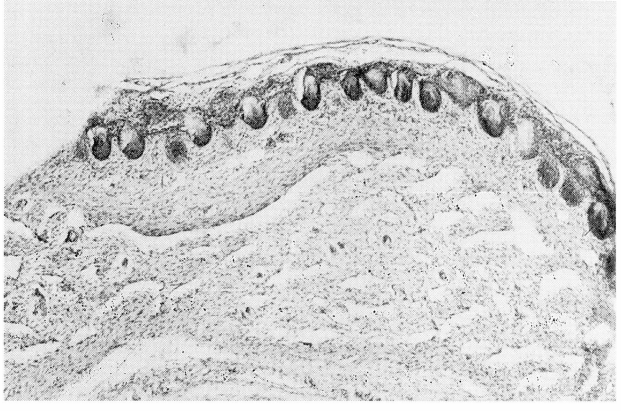
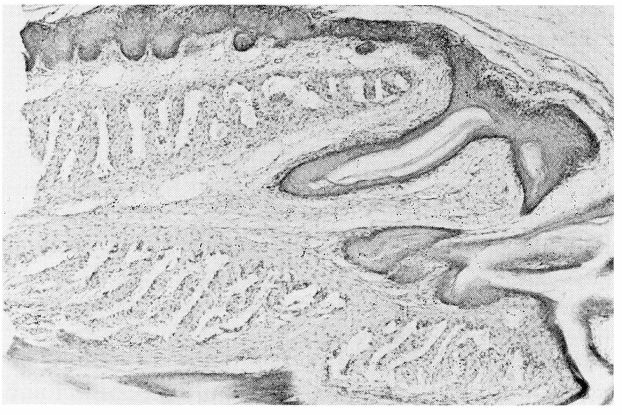


Fig. 4



Figures 1-2. — Normal epidermis and dermis in the control group of rats (H&E x 16 & 41).
Figures 3-4. — Atrophic epidermis and reduced number of hair follicles and sebaceous glands in the first experimental group of rats (H&E x 41).

Table 1. — *Number of births in the control and experimental groups.*

Indices (Dam rats)	Number of litters (Control group)	Number of litters (1st group)	Number of litters (2nd group)
1	7	10	9
2	11	10	8
3	11	7	8
4	9	10	8
5	8	9	7
6	9	8	10
7	8	11	10
8	10	9	10
9	10	8	8
10	8	8	11
Total	91	90	89
Average	9	9	9

Table 2. — *Number of male and female litters in the control and experimental groups.*

Indices (Dam rats)	Number of litters (Control group)		Number of litters (1st group)		Number of litters (2nd group)	
	Male	Female	Male	Female	Male	Female
1	4	3	6	4	3	6
2	7	4	5	5	4	4
3	5	6	2	5	5	3
4	7	2	4	6	5	3
5	3	5	3	6	4	3
6	2	7	4	4	5	5
7	4	4	4	7	6	4
8	5	5	4	5	3	7
9	7	3	3	5	4	4
10	3	5	5	3	8	3
Total	47	44	40	50	47	42
Average	5	4	4	5	5	4

Table 3. — *Mean weight and length of the litters in the control and experimental groups.*

Indices (Dam rats)	Mean weight/length Control group	Mean weight/length Experimental group (1st group)	Mean weight/length Experimental group (2nd group)
1	6.15 g/7.50 cm	4.50 g/5.20 cm	6.15 g/5.50 cm
2	6.35 g/8.00 cm	5.00 g/5.05 cm	6.00 g/5.00 cm
3	6.50 g/7.25 cm	4.75 g/5.00 cm	6.35 g/5.75 cm
4	6.55 g/7.50 cm	4.75 g/5.25 cm	6.15 g/5.25 cm
5	6.25 g/7.75 cm	4.75 g/5.50 cm	6.05 g/5.00 cm
6	6.65 g/7.75 cm	5.00 g/5.25 cm	6.00 g/5.20 cm
7	6.40 g/7.00 cm	4.65 g/5.00 cm	5.65 g/5.25 cm
8	6.75 g/8.00 cm	4.60 g/5.00 cm	6.25 g/5.30 cm
9	6.50 g/6.50 cm	4.50 g/5.15 cm	6.05 g/5.50 cm
10	6.25 g/7.50 cm	4.75 g/5.05 cm	6.15 g/5.35 cm
Mean	6.44 g/7.48 cm	4.73 g/5.15 cm	6.08 g/5.31 cm
S.D.	0.19/0.46	0.18/0.16	0.46/0.23

Table 4. — *Histopathological findings in the experimental group of male rats.*

Histopathological finding	First Experimental Group (n:40)	Second Experimental Group (n:47)
Hyperkeratosis and/or parakeratosis	27	36
Atrophic epidermis	35	44
Atrophic sebaceous glands	36	45
Decreased number of hair follicles & sebaceous glands	36	45

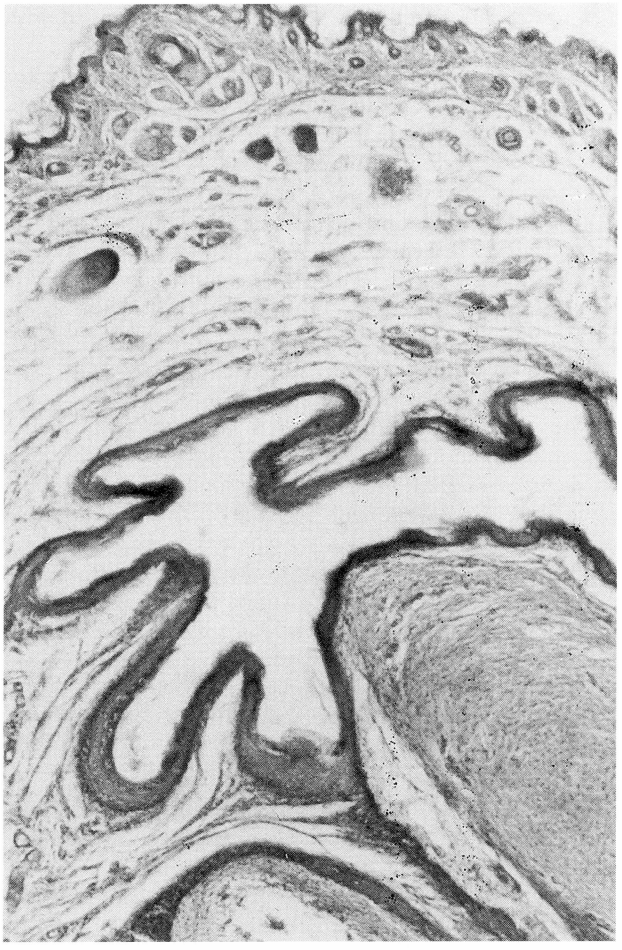


Fig. 5

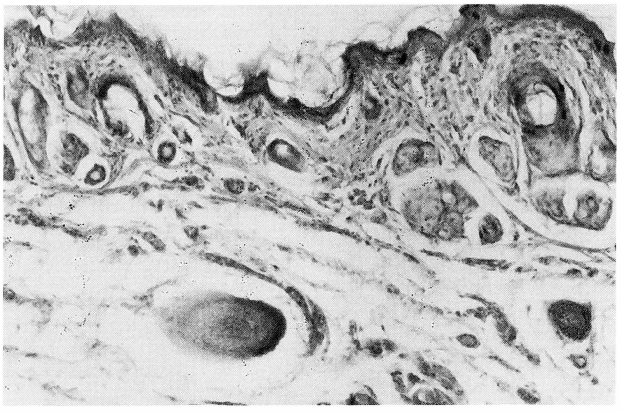


Fig. 6

Figures 5&6. — Prominent atrophy in the epidermis and conspicuously reduced number of hair follicles and sebaceous glands in the second experimental group of rats (H&E x 16 & 41).

germ cells undergoing spermatogenesis, Sertoli cells, peritubular cells and interstitial cells [4].

It has been shown that EGF regulates testosterone production by normal Leydig cells isolated from different species which raises the possibility that EGF may effect spermatogenesis either directly or indirectly through its effects on testosterone production [5-7]. Accordingly, EGF administration was found to reverse the undescen-

ded testes and epididymal abnormalities associated with time specific flutamide administration [8].

In animal studies, a role for epidermal growth factor (EGF) has also been indicated in androgen-dependent male sexual differentiation [9, 10]. Although the mechanism by which EGF modulates male sexual differentiation has not been determined, it appears that testosterone-induced male sexual differentiation is accompanied by an increase in EGF gene expression. It has been also shown that prenatal exposure to the antiandrogen flutamide significantly reduced the immunostaining of the EGF-like protein in the Wolffian duct [11]. Furthermore, EGF administration to pregnant mice resulted in persistent Wolffian ducts in female offspring [8].

The submandibulary gland in the mouse is a rich source of epidermal growth factor and there is at least ten times as much EGF in the submandibular glands of male mice than those of female mice [12]. In our study, the teratogenic effects were investigated in the male offspring of sialoadenectomized and prenatally flutamide administered rats. In a group study, the effect of sialoadenectomy was investigated in eight-week old mice and reduced body weight and reproductive organ weights were observed after sialoadenectomy. We also noted a reduced mean body weight in the litters of sialoadenectomized rats (Table 3). This evidence indicates that EGF is an important mediator of normal growth.

In an animal study, the effects of sialoadenectomy and treatment with EGF antiserum on epidermis were investigated [2]. The thickness of the epidermis in sialoadenectomized mice was found to be significantly reduced. A further decrease in the thickness of the epidermis was also noted after EGF antiserum administration in sialoadenectomized mice. No appreciable change was observed in the dermis and subcutaneous tissue in this study. In our study, an atrophic epidermis was also observed in both experimental groups. However, atrophic dermal adnexa together with a decreased number of sebaceous glands and hair follicles were striking features in the male offspring of the flutamide administered group of rats (Figures 5, 6).

Overall, teratogenic effects of maternal EGF deficiency and flutamide administration were observed histologically on rat skin development. It is our conclusion that epidermal growth factor is a key hormone for skin development and antiandrogen administration may insult this process by interfering with epidermal growth factor metabolism.

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