Effects of clomiphene citrate on neonatal rat skin

E. Deveci[1], H. S. İnalöz[2], S. S. İnalöz[3], B. Ünal[3]

[1]Department of Histology & Embryology, University of Dicle Faculty of Medicine, Diyarbakır
[2]Department of Dermatology, Univeristy of Gaziantep Faculty of Medicine, Gaziantep
[3]Department of Histology & Embryology, University of Gaziantep Faculty of Medicine, Gaziantep (Turkey)

Summary

Background: Clomiphene citrate is chemically related to non-steroidal estrogens, and has antiestrogenic properties. It is used in the treatment of anovulatory female infertility and its therapeutic effect mainly depends on inhibiting the negative feedback effects of endogenous estrogen by stimulating the gonadotropin releasing hormone. Today, it is also used in the treatment of male infertility.

Objectives: In this study the effects of clomiphene citrate on skin maturation in neonatal rats were investigated.

Methods: Forty Spraque-Dawley female newborn rats were separated into two control and two experimental groups (n=10). One day after birth, experimental newborn rats were given clomphene citrate subcutaneously in a dosage of 100 mg/kg/day for five days. The first experimental group of rats were anesthetised at 21 days whereas the second experimental group of rats were then anestetised on day 28. Biopsies were taken immediately from the perineal skin. Histopathological assessments were made and compared with their control groups.

Results: In both the experimental groups of newborn rats, increased keratinization and irregular hypertrophy were observed in the epidermal cells. Disorganization of the basal layer cells and hyperplasia were found to be more prominent in the first experimental group and dermal fibrosis and lymphohistiocytic inflammatory cell infiltration were especially prominent around the sebaceous glands in the second experimental group.

Conclusion: The administration of clomiphene citrate in newborn rats showed impaired skin maturation.

Key words: Clomiphene citrate; Newborn rat; Skin.

Introduction

Clomiphene citrate (CC) is used in the treatment of anovulatory female fertility. It has an anti-estrogen activitiy by occupying estrogen receptors in the hypothalamus and anterior pituitary which induce gonadotrophin-releasing hormone (GnRH) and gonadotropin release. Its use in male fertility has not been proven [1].

With the administration of antiestrogen in male neonatal mice, testicular hypoplasia, intra-abdominal testes, epididymal cysts, and squamous metaplasia of accessory glands, decreased weights of testes, epididymis and seminal vesicle have all been observed. Similar findings, such as decreased weight of the uterus and vagina were also found in the female neonatal mice [2].

The effect of CC administration on skin maturation has not been well documented. The aim of the present study was to assess histologically the effects of neonatal CC administration on skin maturation in rats.

Material and Methods

Forty Spraque-Dawley female newborn rats were separated into two control groups and two experimental groups (n=10). One day after birth, experimental newborn rats were given CC subcutaneously in a dosage of 100 mg/kg/day for five days. The first experimental group of rats were anesthetised at 21 days whereas the second experimental group of rats were anesthetised on day 28. Immediately after, biopsies were taken from the perineal skin. Tissue samples were fixed in a solution of 10% formaldehyde. The tissues were then embedded in paraffin wax, serial sectioned and stained with hematoxylin-eosin and trichrome-masson for evaluation using a light microscope. Histopathological assessments were made on all groups.

Results

Histopathological examination showed increased keratinization and hypertrophic epidermal cells in both experimental groups of rats. Disorganization of the basal layer cells with irregular hyperplasia were more prominent in the first experimental group (Figure 1). Normal arrangement of the basal layer cells and the dermis was observed in the first control group of rats (Figure 2).

Increased epidermal keratinization and lymphohistiocytic inflammatory cell infiltration were found to be prominent around the sebaceous glands in the second experimental group (Figure 3). Normal arrangement of the basal layer cells and the dermis were observed in the second control group of rats (Figure 4). Overall, impaired skin maturation was observed with CC administration in both experimental groups of rats (Table 1).

Discussion

The teratogenic effect of CC has not been well documented. Open neural tube defects have been reported on newborns following the administration of clomiphene citrate to infertile women [3, 4, 5]. Although, it has been

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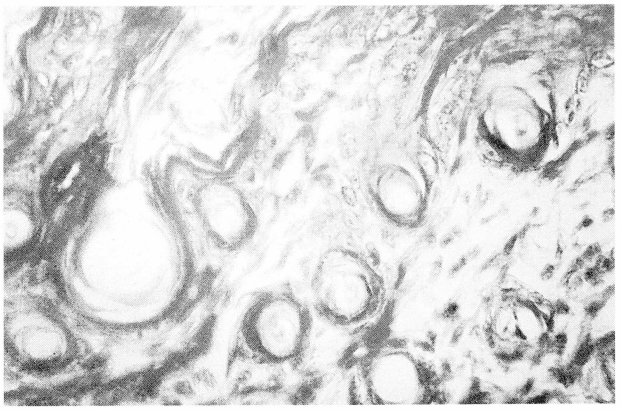

Figure 1. — Hypertrophic epidermal cells and disorganisation of the basal cells in the first experimental group (Trichrome Masson, original magnification x 82)

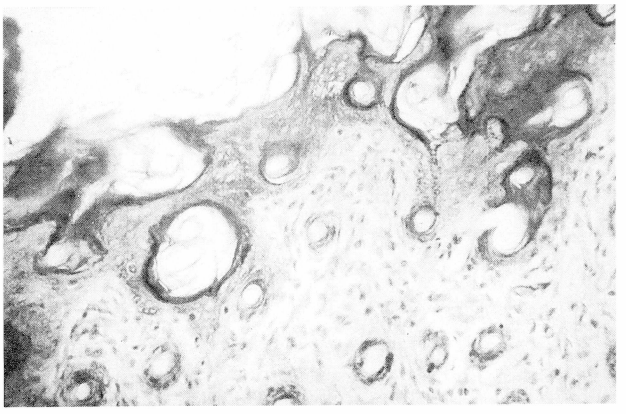

Figure 2. — Normal epidermis and normal arrangement of the basal cells in the first control group (H&E, original magnification x 82).

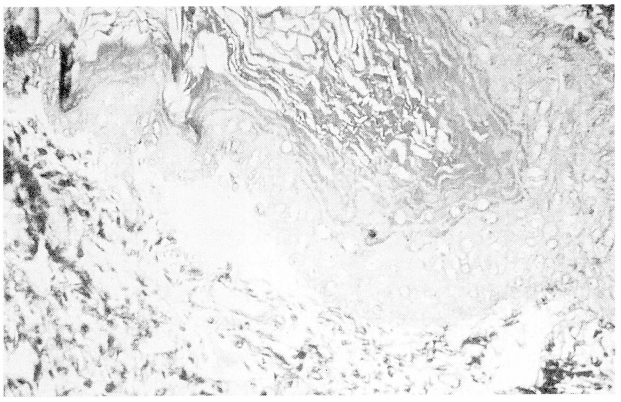

Figure 3. — Increased keratinization and dermal chronic inflammatory cell infiltrate particularly around the sebaceous glands in the second experimental group (Trichrome Masson, original magnification x 82).

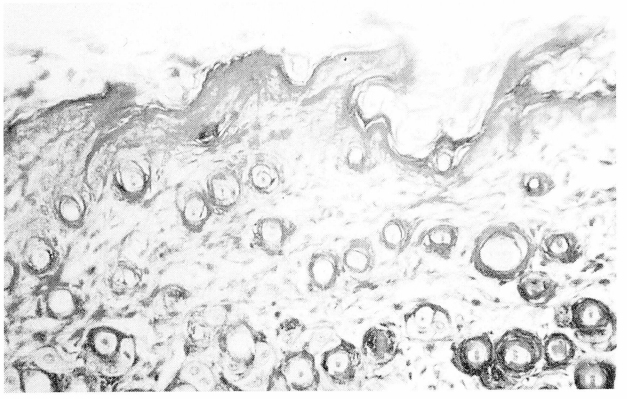

Figure 4. — Normal epidermis and dermis in the second experimental group (Trichrome Masson, original magnification x 82).

Table 1. — *Histopathological findings in the experimental groups.*

Histopathological finding	First experimental group (n:10)	Second experimental group (n:10)
Increased keratinization	10	10
Hypertrophic epidermal cells	10	10
Disorganization of the basal cells	9	5
Irregular hyperplasia of the basal cells	10	6
Dermal inflammatory cell infiltrate	3	9
Dermal fibrosis	2	8

suggested that the incidence of fetal disorders is not increased with CC administration, several abnormalities have been recorded with neonatal antiestrogen administration in mice genitalia. These abnormalities include testicular hypoplasia, intra-abdominal testes, epididymal cysts, and squamous metaplasia of accessory glands [6]. In another study, the weights of the testes, epididymis, and seminal vesicle were found to be significantly lower than in neonatal male controls. Similar findings, such as decreased weight of the uterus and vagina were also observed in the female neonatal mice [2].

In a group study, a great impairment in the development of uterine mesenchymal tissues after CC treatment was observed [7]. The fallopian tube was also affected in that its epithelium was hyperplastic and disorganized. In several studies, epithelial proliferation and superficial cornification were major findings in the uterus and the vagina with CC and tamoxifen administration in animal models [8, 9, 10]. We also observed similar findings in the rat skin of the experimental groups (Figure 3).

It was postulated that the uterine abnormality resulted in a loss of type III collagen and laminin, and in an increase in fibronectin and type I collagen in the mesenchymal stroma of mice treated neonatally with antiestrogens [11]. In our study dermal fibrosis was observed especially in the second experimental group of rats.

In conclusion, we observed histologically impaired skin maturation with neonatal CC administration in rats. The present study suggests, therefore, that the first few days postnatal may well be a critical period for skin maturation.

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Address reprint requests to:
H. S. INALOZ, M.D.
Department of Dermatology
University of Gaziantep
Faculty of Medicine, Kilis Yolu Uzeri
Gaziantep 27310 (Turkey)