Curative effect of TFX-Jelfa supplementation on the skin of ovariectomised rats - morphological study

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Abstract

The aim of this study was to assess the effect of thymus factor X (TFX-Jelfa) treatment in hypoestrogenic female rats for the purpose of decreasing skin impairments. Ovariectomised rats were used as a model. The histopathological analysis of the skin after TFX-Jelfa treatment demonstrated that the epidermis was thicker and more desquamated, without deep wrinkles or hypersecretion in comparison to the skin of animals only castrated and not treated with TFX-Jelfa. Collagen and elastic fibres were arranged more uniformly in the dermis and there were numerous fibroblasts, hair follicles, and small vessels. Ultrastructural analysis showed keratinocytes without degenerative changes and the proliferation of lymphatic and dendritic cells in the skin. The results indicate that thymus extracts can have beneficial effects on skin aging, which is often accompanied by hormonal perturbances.

Keywords: rats, thymus hormones, aging skin, histopathology, ultrastructural examination.

Introduction

The skin serves many important functions in the organism and therefore aging, menopausal, or post sterilisation alterations in its appearance are one of the most worrying changes for humans. Microscopic images of the skin in such cases usually show reduction or enlargement of the thickness of certain layers of the skin, changes in the structure of collagen fibrils, elastosis, wrinkles, hyperpigmentation, hair thinning, reduction in size and number of sebaceous glands, and storage of atypical lipids and cholesterol droplets in the hypodermis (3, 4, 11, 20). It is known that thymus hormones have an effect on skin-associated lymphoid tissue (SALT) (18). They may modulate the function of thymus-derived cells and induce maturation of precursor cells into active T lymphocytes including T-helper cells, cytotoxic T cells, and regulatory T cells (1). The extract of juvenile calf thymuses as thymus hormones enhances production of the interleukin-2 (IL-2), interferon-γ (IFN-γ), and antioxidant enzymes. It was reported that thymus hormones may be helpful in the treatment of many immunodeficiency-related or chronic immune-mediated diseases e.g. rheumatoid arthritis or multiple sclerosis, as well as neoplastic, neurological, and connective tissue diseases and even severe burns and wounds, or viral and parasitic infections (7, 13, 15, 21).

The following study was undertaken to determine the influence of thymus factor X (TFX-Jelfa), an aqueous extract of juvenile calf thymuses, on the ultrastructure of the skin in the ovariectomised rats.

Material and Methods

The experiment was carried out on adult female Wistar rats (11-12 months old), weighing 200 - 350 g. The hypoestrogenic status in the animals was induced by bilateral ovariectomy in the 3rd month of life. The animals were fed a standard laboratory diet and kept in cages with light-dark cycles and allowed free access to water and feed. After a two-week adaptation to the new
environment and diet, the rats were randomly divided into the following 3 groups of 10 animals each: sham-operated control rats (C), rats after bilateral ovariectomy (OV), and rats ovariectomised after intraperitoneal treatment with TFX-Jelfa (PF Jelfa S.A., Finepharm, Poland) at a dose of 9 mg/kg b.w., for 14 d (OV-TFX).

The sham-operation on control rats was performed in the 3rd month of life to show and eliminate the impact of operation stress on the examined parameters. In all groups, operations proceeded under general anaesthesia. After the end of the experiment, the rats were euthanized. The animals were maintained in accordance with the Local Ethics Committee at the University of Life Sciences in Lublin.

Samples for microscopic examination were obtained by skin biopsies from inguinal regions, fixed with 4% glutaraldehyde in a sodium cacodylate buffer, as well as 2% osmium acid, and then embedded in Epon 812. For light microscopy (LM), the sections were stained with methylene blue, for transmission electron microscopy (TEM), ultra-thin sections were contrasted by uranyl acetate and lead citrate, and for scanning electron microscopy (SEM), surfaces of the samples were covered with palladium and gold.

Results

Scanning electron microscopy results. In group C, the surface of skin observed in the SEM was slightly corrugated. Hairs were straight and grew densely. Epidermis and dermis were compact (Fig. 1a). In group OV, the skin surface had deep grooves, hairs were singled and layers of the dermis were stratified (Fig. 1b). In group OV-TFX, there were no deep furrows on the skin surface. The majority of young hairs had different diameters and length. Many of them grew in clumps. At the cross-section, the correct arrangement of collagen fibres below the epidermis was evident (Fig. 1c).

Light microscopy examination results. The histopathological examination of samples from the group C revealed regular layers of the whole skin (Fig. 2a). In the group OV, the thin epidermis (E) had deep furrows with hypersecretion. The basement membrane of E was flattened. There were degenerative cells within the skin and connective tissue. The sweat and sebaceous glands were small and localised in deep layers of the skin. Collagen fibres (CFs) were rarefied and arranged multidirectionally. Numerous elastic fibrils were rigid, fragmented, and scattered deep in the dermis (D) (Fig. 2b). There were visible degenerated nervous fibres and condensed sebaceous glands. In the hypodermis there was evident thin adipose tissue with abnormal lipid cells (not shown).

In the group OV-TFX, all constitutive layers of E were thick but uniform. The surface of the skin was slightly wrinkled without pachynsis of the Stratum corneum as in the group C, but there were more exfoliated cells. The dermis was dominated by many young hair follicles and was rich in small vessels.
Transmission electron microscopy results. TEM examination of the group C revealed that skin was composed of a superficial stratified squamous keratinised epidermis (not shown)—and the papillary layer had many fibroblasts (F) and collagen fibres (Fig. 3a).

In the group OV there was the non-uniform thinned Stratum corneum (not shown). Some epidermis cells exhibited features of necrosis and many of them had numerous vacuoles and degenerative mitochondria with diluted matrices. Tonofilaments on the edges of keratinocytes were rigid and assembled in thick bundles. The basement membrane was flat, and under it there were splitting collagen fibres (Fig. 3b) with decreased mean diameter (not shown).

In the group OV-TFX, the Stratum corneum of the epidermis showed features typical for desquamating stage (not shown) and the Stratum spinosum (S) was thick. Cell nuclei had large nucleoli, and the cell membranes of the prickle cells had long cytoplasmic projections and numerous desmosomes. Tonofilaments were not grouped in bundles but evenly dispersed on the edges of keratinocytes as in group C. There were also visible keratinocytes in a mitotic and apoptotic stage (not shown). Underneath the folded basement membrane, there were numerous active fibroblasts and compact collagen fibres (Fig. 3c) of varying diameter (not shown). There were many lymphocytes and dendritic cells (Langerhans cells) in S (Fig. 3d). In the deeper layer of dermis, numerous small hair follicles with sebaceous glands, sometimes mast cells, expanded blood and lymphatic vessels, and normal adipose tissue arranged into lobules were also noted (not shown).

Collagen fibres were tight and properly aligned throughout the skin (Fig. 2c).
Fig. 3b. The TEM image of the fragment of E from group OV; inside the keratinocytes there are degenerative vacuoles. Tonofilaments are assembled in thick bundles. Under a basement membrane (B→) there are loose collagen fibres (CFs) (4400×)

Fig. 3c. The TEM image of a fragment of E from group OV-TFX; the cells of Stratum spinosum (S) and Stratum basale have long cytoplasmic projections and thin tonofilaments. Among keratinocytes there is the dendritic cell (DC). Collagen fibres (CFs) have a correct arrangement (7000×)

Fig. 3d. The TEM image of the fragment of DC with Birbeck granules (→) in the Stratum spinosum of E from group OV-TFX (20 000×)

Discussion

The aging process is a natural feature of each organism. The skin is an organ dependent on many hormones. Oestrogenic and androgenic receptors are present in the cells of the epidermis, hair follicles, and their associated pilosebaceous structures, and in the apocrine sweat glands. There is evidence that the hypo-oestrogenic stage of the organism causes significant changes in the physical characteristics of the skin and decreases its biomechanical quality (3, 11, 19, 20).

All thymus hormones enhance immunoglobulin synthesis, which leads to a more rapid elimination of infectious agents, and improves the general and local immunity of the organism to disease. They accelerate the processes of tissue regeneration and affect the circulatory system by reducing vascular resistance and acceleration of the heart rate (6, 14, 16). Thymus hormones also act synergistically with somatotropin and oestrogens and are the antagonists of androgens, corticosteroids, and progesterone (10).

TFX-Jelfa was used in the treatment of diseases of different pathogenesis and different clinical course. It was shown that the thymus hormones affected the skin through its participation in the pro- and anti-oxidative reactions, normalisation of lipid level, and stimulation of cytokines in the body (2). The well-known ability of fraction 5 thymosin hormones and thymostymulin to increase expression of lymphokines (IL-2 and interferon-γ), together with antioxidant enzymes, decreases lipo-oxygenation (7).

With age, the repertoire of the immune-competent cells becomes gradually exhausted, inter alia because of a slow involution of the thymus, which should provide these cells (5).

Stanulis-Praeger et al. (17) demonstrated that the extract from calf thymus stimulated the proliferation of keratinocytes in vitro, probably because of morphologic similarities between thymic and dendritic skin cells, but this process depended upon the donor’s age. Our research has shown many positive changes in the skin of hypoestrogenic animals after an application of TFX-Jelfa. There were no deep grooves on the surface of the skin; and neither were hairs of varying thickness singled or weak. There were observed an
increased exfoliation of dead cells in the *Stratum corneum* and numerous keratinocytes in the mitotic or apoptotic stage in the *Stratum germinativum* indicating good regeneration of the epidermis. No excessive secretions on the surface of the skin and smaller number of degenerate or necrotic cells across all skin layers were visible. No short rigid tonofilaments were noted in the border of keratinocytes. There were the uniformly arranged collagen and elastic fibres under the delicate wavy basal membrane. Moreover, the correct morphology of adipocytes, a large number of fibroblasts, blood or lymphatic vessels, and hair follicles with small glands were good indicators of the condition of the skin.

TFX-Jelfa treatment proved to be beneficial for the skin probably through the inhibition of reactive oxygen species production and increasing the activity of the thymus, which was reduced after the removal of ovaries (12). Moreover, it improved the fibril architecture and skin elasticity, and induced an overgrowth of hairs (9). In addition, this compound enhanced the angiogenesis and affected fibroblast activity (16).

In conclusion, TFX-Jelfa would be useful not only in many immune-related diseases but as a therapeutic agent in the treatment of selected dermatologic disorders like hypoestrogenism. Further investigations are needed to determine the proper dosage of this medicament for both humans and animals.

**Conflict of Interests Statement:** The authors declare that there is no conflict of interest regarding the publication of this article.

**Animal Rights Statement:** The experiment was conducted in accordance with the Local Ethics Committee at the University of Life Sciences in Lublin.

**References**