Rat skin as an experimental model in medicine

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ABSTRACT

Scientific experiments due to safety and ethical limitations regarding research human are often based on animal models. Rats are laboratory animals which are commonly used for these purposes. It should be remembered that morphological and functional differences between rat skin and human skin may refer to the correct interpretation of scientific results.

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INTRODUCTION

Despite the differences in the structure of the rat skin and human skin, many experiments on these animals can be made. Thanks to functional similarity to a human skin, it allows to determine the toxicity or benefits of the proposed therapies and medications [1,2].

The skin is one of the largest organs of any living organism, including rats. It is an important element separating the body from the external environment. It should be remembered that it unifies by receiving stimuli and microbial colonization with this environment. The skin is not morphologically uniform structure, it covers the whole body surface and constitutes about 15% of the body weight of the rat [3].

The structure of all mammalians' skin is similar and shows a layered structure. The epidermis, which is the external layer of the skin, is an ectodermal origin. Other layers: dermis, subcutaneous adipose tissue, nerves, musculature and vessels are mesodermal origin [4,5].

Rat skin contains similar enzyme systems, however, this can not be directly related to human skin due to the fact, there are not all enzymes present in both species and the enzimes exhibit different activities. This may result in misinterpretation of the results of biochemical analyzes [1]. Rats can be used as an animal model of a disease and its complications or as an effectiveness of the therapy. Rat skin can be used to assess the impact of nutritional deficiencies or aging [6,7,8].

Epidermis

The epidermis is a multilayer, keratinized, flat epithelium that covers the outer surface of the dermis. The epidermis of the rat, just like in human, consist of 5 layers max. Its thickness, depends on the data and ranges from 13.46 to 167.1µm [4,5,7,8,9,10]. Breed of rat, its age and the animal body surface area makes the difference with the epidermal thickness. The human epidermis average thickness is 100 µm (between 50µm to 1 mm) [9,11,12].

The stratum corneum, is made of dead cells containing keratin. Due to the presence of keratin, the epidermis is a barrier that protects against water loss and penetration of substances from the external environment. The stratum corneum of rat is the thickest on its feet. Adults rats stratum corneum thickness is 165µm [8] and a whole epidermis thickness is between 350 and 450µm [9]. Moreover, there are several layers of dead and flattened cells (corneocytes) of the skin in the back, which closely adhere to each other in the lower layers and are loosely laid on the surface of the epidermis, constantly undergo exfoliation (stratum disjunctum) and constitute about one third of the thickness of the epidermis (13.7- 34.7µm) [10].

On the surface of the stratum corneum, tere is a lipid coat, chich is a mixture of sebum secreted by sebaceous glands and lipids derived from epidermal cells. In addition, the lipid coat includes waxes, fats, fatty acids and hydrocarbons, as well as products of the bacterial flora metabolism of the skin, e.g. the lactic acid. The thickness of the lipid coat is variable at various stages of rats life and largely depends on the influence of sex hormones on the activity of the sebaceous glands. The lipid coat regulates the processes of absorption and penetration into the skin of water-soluble and fat-soluble components. It is important for maintaining proper hydration of the stratum corneum. The renewal of the lipid coat lasts about few hours. The slightly acidic surface of the skin provided by the hydrolipidic coat and physiological flora pH 4.2-5.6 protects against chemical agents, pathogenic bacteria and fungal infections [4,5,7,10,13-17].

The intermediate layer of the epidermis (stratum lucidum or intermediale), formed by tightly adherent keratinocytes, is very thin. This layer is visible in the callused epidermis of the soles of the paws. It owes its name to the optical properties of the protein that builds it, eluidin, considered to be a product of the transition of keratohialin to semi-liquid state [4,5,10,13-17].

Granular layer of the rat skin (stratum granulosum) is clearly marked on the whole surface of the skin. It consists of 2-3 layers of spindle cells filled with keratohialin grains, the intermediate product of the keratin precursor. The cells of the granular layer, also referred as an intermediate layer, are flattened smoothly into the underlying horny layer [4,5,10,13-17].

The stratum spinosum of the rat epidermis is composed of several polygonal cells layers. The cells of the spinous layer do not adhere closely to each other but they are separated by spaces which give a pattern of gaps. The substances that cement individual cells are a mixture of proteins and proteoglycans. The role of intercellular connections is played by desmosomes. They are multilayer thickening of the cell membrane located in the area of adherence of two spinal cells to each other. The spinosum layer is the thickest living part of the human epidermis [11]. This layer in the rat skin is poorly developed and in some parts of the skin even invisible where the basal layer dominates [4,5,10,13-17].

The basal layer of the rat skin (stratum basale), called as a reproductive layer, is the lowest one. It is made up of one layer of cylindrical or cuboidal cells closely adjacent to each other. Among the cells presented in the basal layer we distinguish keratinocytes, in which the process of producing fibrous keratin precursors begins. Keratinocytes constantly multiply and divide and thanks to that process the epidermis is constantly renewed.

Another type of cells in the basal layer are melanocytes, i.e. dendritic cells, responsible for the production of skin pigment - melanin [11]. In the skin of rats, the dye is produced near the anagen hair bulb [9] and not in the basal layer of the epidermis but it is typical for a human skin [11]. In thick parts of the rat's epidermis there are Merkel cells containing structures grouped around larger hair follicles and acting as touch bodies [9]. The cells of the basal layer are connected to each other and to the cells of the spinous layer located above desmosomes, i.e. the protuberances of the cell membrane of adjoining cells. With a basal membrane, basal cells are connected by hemidesmosomes. In this zone there are also integrins (adhesion molecules) that connect cell membranes with elements of extracellular matrix [4,5,10,13-17].

Adhesion of the epidermis to the dermis is ensured by the dermal-epidermal border which consists of a basal membrane and many layers of substances, such as glycoproteins, type 4 collagen or fibronectin. The basal layer together with the dermal-epidermal junction are corrugated, mostly marked in the rat skin exposed to pressure [9]. In the process of aging, the skin warts and the basal membrane disappears and the skin flattens. The thickest epidermis is on the soles of the paws but the thinnest epidermis is on the eyelids [4,5,10,13-17].

Dermis

Dermis is composed of connective tissue containing collagen, giving the skin considerable resistance, as well as suppleness and elasticity [18]. It contains numerous blood vessels and nerve endings, that allows to feel touch or pressure, as well as cold and heat receptors. There are embedded in the skin adnexa - epithelial products such as hair, sebaceous glands, sweat glands and claws. The thickness of the dermis is variable and depends on the area of the body. The thickest skin is covered with the back and the thinnest eyelids. The thickness of the dermis increases with the age of the rat and reaches values from 100μm to 3mm [8,10,15,17].

Two layers are distinguished in the dermis. A papillary layer (stratum papillare) that includes the skin warts containing numerous small blood vessels which can form the sub-dome plexus that nourishes the underlying epidermis. The stratum reticulare, covering the deeper layers of the skin, up to the subcutaneous tissue, is made of more compacted collagen. The connective-tissue subarea consists of three types of fibers: collagen, elastic and reticulin fibers, which are suspended in an amorphous mass of proteoglycans. Collagen fibers type 1 and 3 are parts of the stroma [9]. Type 4 collagen and laminin are found in the basal membranes of the vessels and epithelial basement membrane. Reticulin fibers are found mainly in dermal papillae, while elastic fibers (made of elastin) are intertwined with collagen fibers and are responsible for the elasticity and extensibility of the skin [4,5,10,13-17].

The papillary layer is the most superficially lying layer of human skin and consists of small skin domes raised to the epidermis. The papillary layer consists of very thin, collagen, elastic and reticulin fibers joined together with the capillaries ascending to the top of the warts. In the papillas of the skin are also present nerve fibers, tubes that lead sweat glands and hair follicles. The free spaces between them are filled with the basic substance and fibroblasts, histiocytes and mast cells. The papillary layer is the most metabolically active layer of the skin, which is manifested by its rich vascularity and the abundance of cells involved in the regeneration processes. This layer gradually passes into the reticular layer, with a different pattern and thicker fibrous structure [4,15,16]. In the rat skin, we do not distinguish such a divisible division of the dermis and its structure is more homogeneous. In addition to fibroblasts, T lymphocytes, dendritic cells and mast cells are present in the skin [9].

The dermis contains as much as 80% of water mainlly associated with proteoglycans, while in the epidermis this value decreases to 10-13% in the stratum corneum. Structural proteins are produced by fibroblasts or fibrocytes. These are: the durability of collagen (a macromolecule that combines into fibers) and elastin responsible for skin elasticity. Oxytalane fibers, a special kind of elastic fibers, placed transversely to the right elastic fibers, are very delicate and disappear as the first in the aging process of the skin. They are found mainly in the dermis of the dermal dermis, as well as the reticular fibers, which are very thin collagen fibers. The reticular layer contains mainly collagen fibers and proteoglycans. Cells, mainly fibrocytes or fibroblasts, spindle cells, produce proteins and proteoglycans. In addition to fibroblasts, which are solid cells, the dermis contains immigrating cells of the immune system, macrophages, lymphocytes, granulocytes, mast cells and eosinophils [4,5,10,13-17].

Another layer of rat skin is the subcutaneous tissue (stratum adiposum), which is not clearly defined, but has a looser structure of collagen fibers than the dermis. In the spaces between the fibers there are fat cells (adipocytes). Adipocytes are formed from preadipocytes, cells similar in shape to fibroblasts, but filled with triacylglycerols. Preadipocytes, initially spindle-shaped, gradually lose this shape, round off and transform into adipocytes. Fat cells give the subcutaneous tissue its insulating properties and constitute a backup material. Flabby structure of the subcutaneous tissue of the rat allows the accumulation of a significant amount of water [3-5,10,13-17].

Unlike a human skin, rat skin has an additional layer called panniculus. It is a layer of muscle differentiated in terms of the amount and thickness of muscle fibers. In females, panniculus sometimes appears as a layer of dividing cells, especially in the area where the tissue transforme into mammary glands. The muscle is interrupted by the occurrence of fat tissue foci containing bundles of connective tissue, in which vessels and nerves are found [4,14-16].

The fibrous layer (stratum fibrosum), also absent in human skin, forms the lower boundary of subcutaneous tissue. It consists of small collagen bundles suspended in connective tissue with the consistency of mucus. Vessels (mainly microvessels) and nerves are easily identifiable here. Collagen fibers can be densely packed into single bundles in stratum fibrosum or can be separated by adipose tissue [4,5,16].

The skin of the rat on the ridge reaches an average thickness between 0.65mm and 3 mm [7,8, 10,17].

The function of the skin

The skin is one of the most important organs of the living organism and performs various functions which in a rat do not differ significantly from the function in human.

In mammals, the skin ensures the integrity of the organism with the external environment. An important role of the skin is protection against the influence of adverse factors of the external environment. A dense barrier is the stratum corneum of the epidermis and the constantly occurring regenerative changes ensure its continuity. The outer layers of the epidermis are colonized by bacterial microflora which protects the body against pathogens and the penetration of substances from the external environment, as well as against the loss of water. This process ensures the homeostasis of the system. The skin is resistant to mechanical injuries due to its elasticity and stretch. The presence of fat in the subcutaneous tissue is absorbed by excessive external pressure. In the case of rats, a brown fat is the main fat tissue and it is involved in the thermoregulation process [9].

The skin is the essence of the immune system and is responsible for specific and nonspecific defense reactions. Several skin cells, e.g. Langerhans cells and keratinocytes, have the ability to capture and present antigens [4,11,13,15,19].

The presence in the skin of numerous nerve endings receiving and conducting stimuli: pressure, touch, pain and temperature gives the skin a role of a specialized sense organ. The skin acts as an organ of the senses and remains in close communication with the whole system [11,12].

The skin is involved in the thermo-regulation of the body. Heat from the body is transmitted by radiation, conduction and convection. This regulation is provided by the vascular system of the skin. Which in the case of human skin is divided into a sub-epidermal and deep vessel system. This is important for thermoregulation of the body [9]. At high ambient temperature, the skin vessels dilate and increased blood flow allows the loss of heat stored in the body. When the temperature decreases, the vessels narrow down to counteract heat loss [11,12]. In the case of exhaustion of the possibility of heat loss on the aforementioned ways, the body begins to use the most efficient mechanism consisting in the release of sweat on the skin surface and its evaporation. In this way, the body is at the beginning to adapt to temprature higher than the temperature of the body. Rat as a warm-blooded animal must also equalize the body's temprature. In the case of these animals, sweat sweeps are only on the surface of the foot pads [9]. The basic mechanism is to lower the temprature by increasing the blood flow in the skin and heat loss through radiation and conduction through the vasodilation of the vessels [20,21]. If this mechanism is insufficient, the rat has higher blood flow through the tail, resulting in heat loss of up to 40% heat of basic metabolism [21,22]. In addition, the mechanisms regulating the body temperature of the rat are evaporation from the respiratory tract, skin salivation and micturition [21].

The sebaceous glands of the skin secrete a specific fatty secretion called sebum. The sebum mixed with sweat creates on the surface of the body a thin layer of oil-water emulsion that protects the skin against the harmful effects of chemical agents and bacterias. Sebum oil the surface of the epidermis. In the skin, the lamellar coronal granular layer forms hydrophobic lipid complexes limiting the loss of water from the body [9]. Lubrication of the skin protects it to certain extent against mechanical damage because it plasticizes the epidermis by retaining water in it. The acidity of the epidermis is caused by the breakdown of triacylglycerols to free fatty acids by the bacterial flora of the skin. Acidification of the epidermis also hinders the multiplication of pathogenic microorganisms [12, 23-26].

The skin is involved in metabolic mechanisms in the body. Under the influence of the ultraviolet radiation vitamin D3 is formed, which is mainly responsible for the calcium distribution of the human body [12,27,28]. In the case of rats, the main source of Vitamin D is its supplementation with a food, which is related to the animal diet. Nevertheless, the data from the literature also indicate the possibility of this synthesis in the rat skin under the influence of ultraviolet radiation [29, 30,31].

Although the skin is a tight barrier, it can absorb some chemical compounds from its surface, such as fat-soluble vitamins and certain hormones, eg. used for therapeutic purposes [12].

Conflicts of interest

The author declare that there are no conflicts of interests regarding the publication of this study.

REFERENCES

1. Oesch F, Fabian E, Guth K, Landsiedel R. Xenobiotic‑metabolizing enzymes in the skin of rat, mouse, pig, guinea pig, man, and in human skin models. Arch Toxicol 2014 Dec; 88:2135–90.
2. Knaś M, Niczyporuk M, Zalewska A, Car H. The Unwouned Skin Remodeling in Animal Models of Diabetes Types 1 and 2. Physiol Res 2013;62:519-26.
3. Davies B, Morris T. Phisiological Parameters in Laboratory Animals and Humans. Pharm Res 1993 Jul;10(7):1093-94.
4. English KB, Munger BL. Pathobiology of the Aging Rat. Vol 2. Washington, DC: ILSI Press; 1994. Chapter 4, Integumentary system and mammary gland-Normal development of the skin and subcutis of the albino rat; p363-390.
5. Elwell ME, Stedham MA, Kovatch RM. Pathology of the Fischer Rat. Reference and Atlas. Academic Press; San Diego, London 1990 Chapter 19, Skin and subcutis; p261-278.
6. De Castro E, Boyd. Organ Weights and Content of Rats Fed Protein Deficient Diets. Bull. WldHlth Org 1968;38:971-77.
7. Zaki SM.Characteristics of the Skin of the Female Albino Rats in Different Ages: Histological, Morphometric and Electron Microscopic Study. J Cytol Histol 2015, S:3.
8. Thomas JR. Effects of Age and Diet on Rat Skin Histology. The Laryngoscope 2005 Mar; 115(3):405-11.
9. Sundberg JP, Booth CJ, Flecman P, King Jr LE. London: Academic Press an imprint of Elsevier; 2018 Chapter 24, Skin and Adnexa. P.511-40.
10. Bronaugh RL, Steward RF, Congdon ER. Differences in permeability of rat skin related to sex and body site. Journal of the Society of Cosmetic Chemists 1983;34:127-35.
11. Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. European J Dermat 2002 Jul-Aug;12(4):390-99.
12. Braun-Falco O, Burgdorf WH, Plewig G Dermatologia.[Dermatology] Lublin: Wydawnictwo Czelej; 2011. p. 5-7, 113-4, 594, 885, 1578-81 (Polish)
13. Bacha WJ, Wood LM. Color atlas of veterinary histology. Philadelphia: Lea and Febiger; 2000. p.85-95.
14. Fox J, Anderson L, Loew F, Quimby F. Laboratory Animal Medicine. San Diego: Academic Press; 2002. p121-65.
15. Mousa AM. Histological and immune-histochemical study on the effect of tretinoin on the thin skin of adult male albino rat. Egyptian Journal of Histology 2008;31:208-19.
16. Wells MY, Voute H, Bellingard V i wsp. Histomorphology and vascular lesions in dorsal rat skin used as injection sites for a subcutaneous toxicity study. Toxicol Pathol 2010 Feb;38(2):258-66.
17. Ngawhirunpat T, Hatanaka T, Katayama K, Yoshikawa H, Kawakami J, Adachi I. Changes in electrophysiological properties of rat skin with age. Biol Pharm Bull 2002 Sep;25(9):1192-6.
18. Karimi A, Rahmamati SM, Navidbakhsh M Mechanical characterisation of the rat and mice skin tissues using histostructural and uniaxial data.Bioegineerined 2015 May-June; 6(3):153-60.
19. Reinke JM, Sorg H. Wound repair and regeneration. Eur Surg Res 2012;49:35-43.
20. Gordon CJ. Thermal biology of the laboratory rat. Physiol Behav 1990;47:963-91;
21. Warner SP, Primola-Gomes TN, Pires W, Guimaraes JB, Hudson ASR, Kunstetter AC, Fonseca CG, Drummond LR, Damasceno WC, Teixeira-Coelho F. Thermoregulatory responses in exercising rats: methodological aspects and relevance to human physiology. Temperature (Austin). 2015 Dec 30;2(4):457-75.
22. Young AA, Dawson NJ. Evidence for on-off control of heat dissipation from the tail of the rat. Can J Physiol Pharmacol 1982;60:392-8.
23. Wojas-Pelc A, Nastałek M, Sułowicz J. Estrogen and skin - slowing down the aging process.] Przegl Menopauz 2008;6:314-8. (Polish)

24. Sobjanek M, Zabłotna M, Sokołowska-Wojdyło M, Niedoszytko B, Michajlowski I. Genetic factors in the etiopathogenesis of acne vulgaris. Post Dermatol Alergol 2007; 24(4):183-7. (Polish)
25. Park H, Skopit S. Safety considerations and monitoring in patients treated with systemic medications for acne. Dermatologic Clinics 2016;34:185-93.
26. Rolka H, Lewko J, Kuprianowicz M, Łukaszuk C, Krajewska-Kułak E, Baranowska A, Jankowiak B, Niczyporuk W, Niczyporuk M. Inuence of acnelesions on selectedquality of life parameters] Dermatol Klin [Clinical 2008;10:15-9. (Polish)
27. Dixon KM, Tongkao-On W, Sequeira VB, Carter SE, Song EJ, Rybchyn MS, Gordon-Thomson C, Mason RS. Vitamin D and death by sunshine. IJMS 2013;14:1964-77.
28. Lehmann B, Meurer M. Vitamin D metabolism. Dermatol Ther 2010 Jan-Feb; 23(1):2-12.
29. Schachter D, Finkelstein, Kowarski S. Metabolism of Vitamin D. I. Preparation of Radioactive Vitamin D and Its Intestinal Absorption in the Rat. J Clin Invest 1964 May;43:787-96.
30. Chang E., Kim Y.: Vitamin D Insufficiency Exacerbates Adipose Tissue Macrophage Infiltration and Decreases AMPK/SIRT1 Activity in Obese Rats Nutrients 2017 Mar; 29:9(4). pii: E338
31. Takada K. Formation of fatty acid esterified vitamin D3 in ratskin by exposure to ultraviolet radiation. J Lipid Res 1983 Apr;24 (4):441-8.