Evaluation of a Skin Carcinoma Xenograft Mice Model Supplemented by Chemical Immunosuppression

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As we know today, cancer is a disease caused by the uncontrolled growth of a single cell. This development is triggered by mutations, changes in DNA that affect genes in such a way that they increase cancer cells. In the present study, an experimental model based on skin carcinoma xenograft mice was developed and tested. There were studied: the mice weight, the tumor volume, and the evolution of different skin parameters such as transepidermal water loss (TWL), erythema and the hydration level of stratum corneum; mice were divided in four groups: a control group, a group of mice injected with physiological saline solution, another one with immunosuppression, and a group of mice with immunosuppression and xenograft of A375 cells. The results indicate that administration of cyclophosphamide significantly reduced the number of neutrophils and lymphocytes and last two mice groups revealed more pronounced modifications of skin parameters compared to first two groups.

Keywords: cyclophosphamide, erythema, skin carcinoma, stratum corneum, TWL

Cutaneous cancer, especially malignant melanoma, is one of the most devastating types of cancer.The incidence of skin cancer is increasing even though early prevention and diagnosis has helped to combat this disease.

The importance of this pathology in current medical practice is reflected in research in the field, causing us to seek to understand in detail the mechanisms of producing these tumors so that we can then manage to prevent and treat them [1].

Animal models are used to investigate the molecular, cellular and physiological mechanisms of human disease, to test potential therapeutic substances to characterize physico-chemical and pathological responses to toxic drugs and chemicals, and to confirm or elucidate the precise role of the various factors that cause the disease. Animal models may be graded according to the level at which they were altered compared to the original phenotype and / or genotype. These categories include: (1) natural patterns, (2) manipulated patterns, and (3) genetically modified patterns [2]. Mouse models have made a decisive contribution to progress in important areas of dermatology such as immunology and cancer. In the case of cancer, we should mention the major contributions of the models of skin cancer induced by chemical carcinogenesis, with high impact regarding tumor development, following the typical steps initiation, promotion and progression. This model provides an experimental system of analysis of the molecular mechanisms involved in these stages. Murine cancer models designed to capture the complexity of human cancer currently offer the most advanced preclinical opportunity to study different mechanisms which provides reason for therapeutic development [3].

A variety of approaches for mouse cancer modeling are now available (fig. 1), which presents a number of

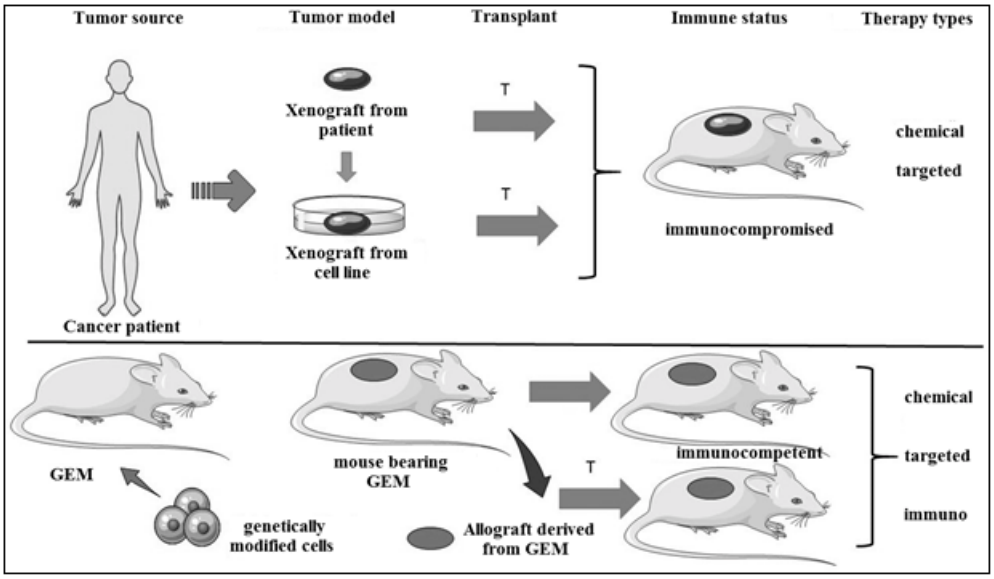

Fig. 1. The current state of preclinical cancer models [5]

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advantages and disadvantages. Thus, the limitations of standard patterns by xenograft derived from the cell line are discussed, models of genetically and biologically engineered mouse cancer are described,and also patterns derived from patient xenografts, reviewing the values and constraints and highlighting recent developments [4].

The skin, the largest organ of the human body, performs a variety of functions. The measurement of skin pH isused in clinical and preclinical research, to evaluate dangerous changes in *p*H after outdoor exposure and to assess the condition of the disease with acute or chronic changes. A balanced *p*H level protects the skin against pollution, prevents infections, irritations, slows the aging process and keeps the skin shiny and healthy.

The loss of transepidermal water (TWL or TEWL) is a term associated with dermatology. It is defined as measuring the amount of water that passes from the inside of the body (human, animal or plant) through the epidermis (skin) to the outside of the body. The loss of transepidermal water occurs through diffusion and evaporation processes, this process is continuous and cannot be controlled by the human body. TWL in mammals is known as "insensitive water loss" because it is a process over which organisms have little physiological control. TWL measurements may be useful for identifying skin lesions caused by certain chemical substances, physical strokes or pathological conditions such as eczema, because the TWL rates increase proportionally to the damage level and favor skin drying. Therefore, transepidermal water loss becomes a significant factor in dehydration associated with several major disease states [6].

In order to understand as accurately, concretely and rapidly as possible the mechanisms involved in these diseases, laboratory animals, especially mice are used. The human skin structure compared to the mouse was analyzed in detail in order to make the most accurate passage from preclinical experiments to clinical. Separation of the epidermis of the dermis is made by a membrane that is not very broad and has a sinuous appearance. Derm is about 90% of the skin's thickness, having a high content of both collagen and elastin, which are secreted by fibroblasts, thus giving the skin its elastic properties. The dermis is separated from the lower tissues by a layer of adipocytes, whose fat accumulation has a damping effect (of pillow).

The structural characteristics of the skin in laboratory animals, such as mice, rats, guinea pigs, rabbits, differ from those of human skin, e.g.: these species have a skin with a thinner epidermis, the dermo-epidermal junction is relatively flat without the sinuous appearance, a structurally vaguely organized dermisand a rudimentary dermal system [7].

Mouse pairs exhibit a collision of hair follicles, while human skin has very large interfolicular areas, with a very rare occurrence of pilose follicles. The human skin has a thick epidermis (with many layers of cells) and a dermis thicker than the mouse skin, and is characterized by downward projections of epidermal ridges reaching the dermis as well. This characteristic is more prominent in psoriasis and correlates with elongation of the dermal papillae, which is known as papillomatosis, being sometimes confused with follicular hyperplasia that occurs in mouse models with inflammatory skin diseases. The most common types of immune system cells in the human epidermis are Langerhans cells and CD8-positive T cells. In the mouse epidermis there is a prominent population of epidermal dendritic T cell type Vy5, which are absent in the human epidermis. Both the human and mouse derms are populated with macrophages, mastocytes, conventional $\alpha\beta$ T cells, and a small population of inborn lymphoid cells. In mouse skin there is an important contribution of $\gamma\delta$ T cells responsible for immune surveillance and production of interleukin-17 [8].

Experimental part

Obtaining of experimental model

Adult, female, Balb/c mice, aged 10-12 weeks were purchased from Charles River (Sulzfeld, Germany). They were kept in standard conditions: light-dark cycles, food and water *ad libitum*, almost constant temperature (25°C) and humidity (60%) and were divided into four groups (5 mice/group) as follows: Group 1 - control group; Group 2 - injected with physiological saline solution (solvent for cyclophosphamide); Group 3 - with immunosuppression; Group 4 - with immunosuppression and xenograft of A375.

Cyclophosphamide used in this research to induce immunosuppression is a powder that can be dissolved in physiological saline solution. Thus, cyclophosphamide was administered in 3 doses IM (final concentration: 180 mg/kg mouse body) as follows: 5 days prior to tumor cell inoculation, 3 days before inoculation and one day before inoculation of tumor cells.

The IM cyclophosphamide solution and the dosages were calculated according to the protocol, and then the subcutaneous tumor cells were inoculated. The protocol involves the following steps: (a) mice were weighed before each administration of cyclophosphamide solution; (b) the doses and the volume of solution required for injection were calculated; (c) the solution was injected on the days provided, at the same time; (d) subcutaneous A375 tumor cells ($1x10^7$cells/mouse) were inoculated. The weight of the mice was also monitored after the last dose of cyclophosphamide as well as throughout the study (immunosuppression is associated with weight decrease).

Measurement of skin parameters

Biochemical and physiological parameters of the skin were monitored: transepidermal water loss (TWL), erythema and skin hydration level were conducted using a Multiprobe Adapter System (MPA5) from Courage-Khazaka (Koln, Germany). The determinations of skin parameters were performed in triplicate at the same moment of the day, by the same operator, in a narrow range of temperature (25°C) and air humidity (55%).

The research was studied and approved by the Ethics Committee from Victor Babes University of Medicine and Pharmacy Timisoara.

Statistics

The measurements of this study were done in triplicate for each determination and the results appear as mean ± standard error. One-way Anova and post-tests were used to determine the statistical difference between Groups 2, 3, 4 and the control Group 1, according other studies of our research team [9-12]. $p<0.05$ was considered statistically significant; *, ** and *** indicate $p<0.05$, $p<0.01$ and <0.001.

Results and discussions

Cyclophosphamide was subcutaneously injected in animals of Group 3 and 4 using a 180 mg/kg mouse body on days 5, 3 and 1 before the tumor cells were injected. Administration of cyclophosphamide significantly reduced the number of neutrophils and lymphocytes in the animals of these groups. Moreover, none of the mice showed signs of toxicity or premature death due to the medical treatment.

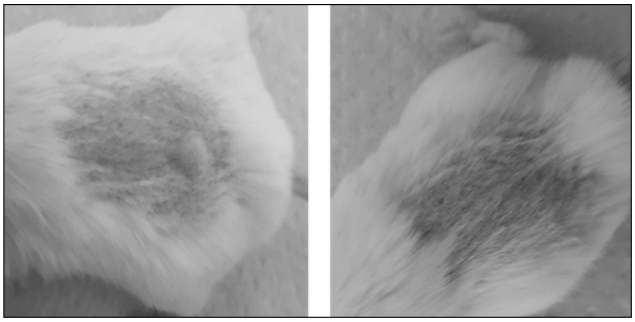

Fig. 2. The appearance and resorption of bubble post-inoculation A375 cells in mice subjected to immunosuppression

Table 1

THE EVOLUTION OF WEIGHT DURING THE EXPERIMENT IN BALB C MICE USED AND DIVIDED INTO THE 4 GROUPS

Day no.	Mean weight [g] / group			
	Group 1	Group 2	Group 3	Group 4
1	24.04	22.22	21.00	23.55
3	24.56	22.34	23.25	20.31
6	24.33	22.13	20.84	20.54
8	23.43	21.66	19.39	20.45
10	24.40	22.90	19.71	21.23
13	20.36	22.71	22.13	19.50
15	23.85	22.91	20.60	19.75
17	24.62	22.96	19.58	17.95
20	23.28	20.76	19.46	19.87
22	24.20	22.49	20.45	19.82
24	24.06	22.32	21.38	20.47
27	23.55	22.14	20.51	21.84
29	24.28	22.13	20.69	19.95
31	24.22	21.64	20.51	21.79
34	25.02	22.06	21.33	22.19
36	24.40	22.25	21.15	20.70
38	24.72	22.41	21.20	21.91
40	25.45	22.56	22.41	22.05

Approximately 1x10^7 cells from each cell line were subcutaneously injected in mice of Group 4. In all injected animals, a palpable and visible bubble was detected on the third day after tumor injection with a 100% absorption rate (fig. 2).

It can be seen from the graph (fig. 3) that the mean tumor volume increased radically every week until the 20th day. Subsequently, tumor volume was shown to increase steadily until the 35th day. By the end of the experiment, tumor volume began to decline in some animals involved in the study.

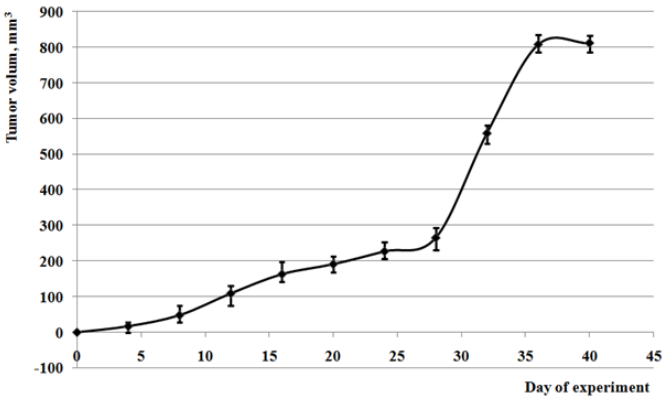

Fig. 3. Evolution of tumor volume during the experiment

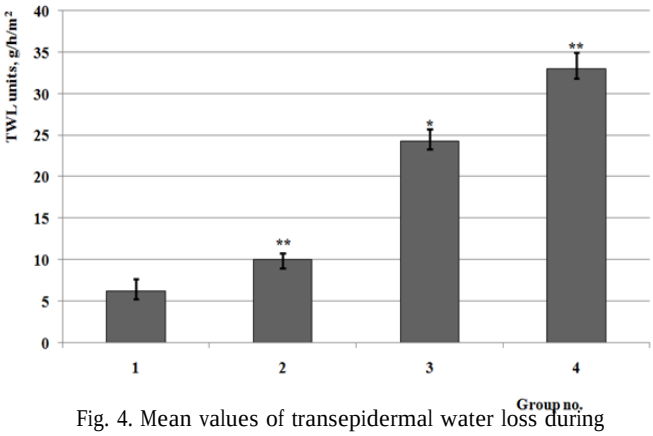

Fig. 4. Mean values of transepidermal water loss during experiment in the 4 groups

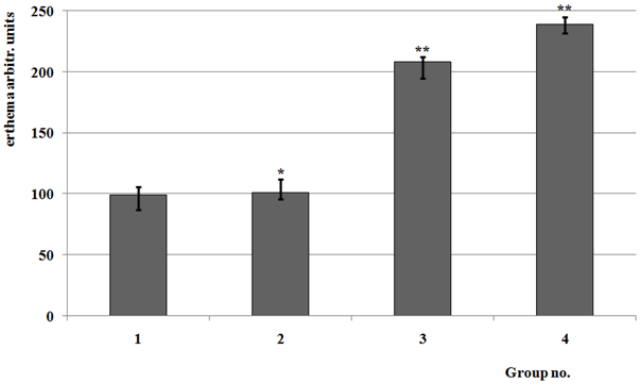

Fig. 5. Mean values of erythema during experiment in the 4 groups

The evolution of weight over time during the experiment is shown in table 1.

Non-invasive measurements of skin parameters

During the experiment, there were no major changes in values of transsepidermal water loss for Groups 1 (control) and Group 2 (injected with physiological saline solution): TWL values indicate a very slight increase that is specific for all compounds with no harmful effects. In Group 3 mice (immunosuppressed), the increased transepidemic water loss was more pronounced than in the case of those inoculated with tumor cells (fig. 4).

In the case of erythema measurements, significant differences were recorded between the first two groups on the one hand and groups 3 and 4 on the other. Values were altered by 8 arbitrary units (Group 1) and 12 arbitrary units (Group 2), while important changes, 51 arbitrary units (Group 3) and 65 arbitrary units (Group 4) were recorded after 40-days experiment (fig. 5).

Another physiological parameter of the skin studied in the present experiment was the water content of the *stratum corneum*, also known as skin hydration. In this case, a decrease in groups 3 and 4 was observed, more pronounced compared to groups 1 and 2 (fig. 6).

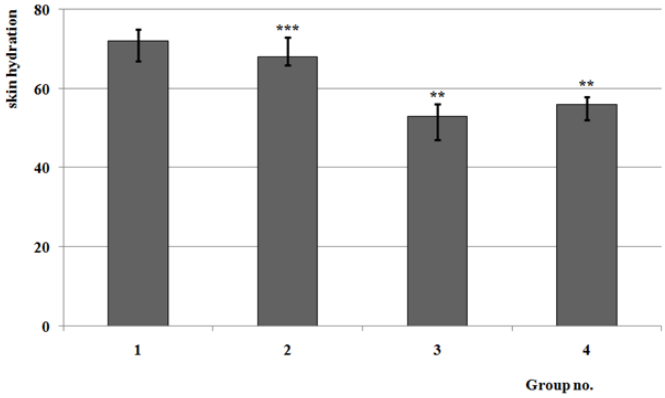

Fig. 6. Mean values of skin hydration during experiment in the 4 groups

Conclusions

Skin cancer affects one in three people who are diagnosed with cancer worldwide, and carcinoma - a malignant tumor of epithelial cells - is the most common type of skin cancer and the easiest to treat if it is diagnosed quickly and right. This paper describes a research based on a new experimental model of skin carcinoma xenograft at mice, supplemented by chemical immunosuppression of cyclophosphamide. Adult female, Balb/c mice were the subject of this study and they were divided in different groups: (1) control group; (2) injected with physiological saline solution; (3) with immunosuppression; (4) with immunosuppression and xenograft of A375 cells. It was found that cyclophosphamice not present any signs of toxicity or premature death; the tumor volume increases very much in the fifth week of experiment and the values of skin parameters (TWL, erythema and skin hydration) were significantly modified in the case of last two mice groups compared to the values obtained in the case of first two mice groups.

References

1. SOICA, C., DANCIU, C., SAVOIU-BALINT, G., BORCAN, F., AMBRUS, R., ZUPKO, I., BOJIN, F., CORICOVAC, D., CIURLEA, S., AVRAM, S., DEHELEAN, C.A., OLARIU, T., MATUSZ, P. Int. J. Mol. Sci., **15**, 2014, p. 8235.
2. WOLF, J.C. Alternative Animal Models. In: HASCHEK, W.M., ROUSSEAUX, C.G., WALLIG, M.A. (Eds.) Haschek and Rousseaux's Handbook of Toxicologic Pathology, 3rd Edition, Cambridge, Massachusetts: Academic Press. 2013.
3. VAN DYKE, T., JACKS, T. Cell, **108**, 2002, p. 135.
4. MINDA, D., PAVEL, I.Z., BORCAN, F., CORICOVAC, D., PINZARU, I., ANDRICA, F., MORGOVAN, C., NITA, L.D., SOICA, C., MUNTEAN, D., TOMA, C.C. Rev. Chim. (Bucharest), **66**, no. 3, 2015, p. 373.
5. DAY, C.P., MERLINO, G., VAN DYKE, T. Cell, **163**, 2015, p. 39.
6. FLUHR, J.W., ELSNER, P., BERARDESCA, E., MAIBACH, H.I. Bioengineering of the skin: water and stratum corneum, 2nd Edition, Boca Raton, Florida: CRC Press. 2004
7. MATHUR, S., KAUR, P., SHARMA, M., KATYAL, A., SINGH, B., TIWARI, M., CHANDRA, R. Phytomed, **11**, 2004, p. 452.
8. SIMOES, M.C., SOUSA, J.J., PAIS A.A. Cancer Lett., **357**, 2015, p. 8.
9. OPREAN, C., ZAMBORI, C., BORCAN, F., SOICA, C., ZUPKO, I., MINORICS, R., BOJIN, F., AMBRUS, R., MUNTEAN, D., DANCIU, C., PINZARU, I.A., DEHELEAN, C., PAUNESCU, V., TANASIE, G. Pharm. Biol., **54**, 2016, p. 2714.
10. TRANDAFIRESCU, C., LEDETI, I., CORICOVAC, D.E., SOICA, C.M., PINZARU, I., DEHELEAN, C.A., IACOB, R.E., BORCAN, F. Mat. Plast., **53**, no. 2, 2016, p. 205.
11. OPREAN, C., BORCAN, F., PAVEL, I., DEMA, A., DANCIU, C., SOICA, C., DEHELEAN, C., NICU, A., ARDELEAN, A., CRISTEA, M., IVAN, A., TATU, C., BOJIN, F. In Vivo, **30**, 2016, p. 633.
12. MUNTEANU, M.F., ARDELEAN, A., BORCAN, F., TRIFUNSCHI, S.I., GLIGOR, R., ARDELEAN, S.A., CORICOVAC, D., PINZARU, I., ANDRICA, F., BORCAN, L-C. Curr. Drug Delivery, 2017, [Epub ahead of print]

Manuscript received: 19.02.2017