DETERMINATION ON CELL CULTURES OF THE PROLIFERATIVE ACTION OF SOME SEMISOLID PREPARATIONS FOR TOPICAL USE WITH AMYGDALI OLEUM

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Abstract

The objective of the study was to evaluate the the cellular proliferation and cell morphology of the gel with *Amygdali Oleum* var. dulcis (sweet almond oil) on cell cultures.

The testing was performed using a line of human lung fibroblasts (MRC), cultivated on a Minimum Essential Medium (MEM) culture containing 10% fetal serum and antibiotics. The MTT test [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] for cellular proliferation was performed after 24 hours, 48 hours and 72 hours incubation of the cells in the presence of the tested substances. We tested semisolid preparations for topical use containing 5% *Amygdali oleum*. Cicatrizin® was used as reference. Cellular proliferation values increased in direct proportion with the cultivation time. At concentrations of 25 mg/mL and 50 mg/mL, the cellular proliferation was higher than for the 10 mg/mL concentration. The human fibroblasts proliferation rates in the presence of sweet almond gel and of Cicatrizin® were comparable with those of the control sample.

Electronic microscopy images showed that the tested products did not induce morphological modifications of the cultured fibroblasts. After 72 hours of incubation, the cells maintained the phenotype and characteristics of normal fibroblasts.

Rezumat

Studiul a urmărit evaluarea acţiunii proliferative şi a morfologiei celulare induse de gelul cu *Amygdali Oleum* var. dulcis (ulei de migdale dulci) în culturi de celule.

Testarea a fost efectuată pe o linie de fibroblaste din plămân uman – MRC, cultivate în mediu de cultură MEM conţinând ser fetal 10% şi antibiotice. Testul MTT de determinare a proliferării celulare a fost realizat după 24 de ore, 48 de ore şi respectiv 72 de ore de incubare a celulelor în prezenţa substanţelor testate. Pentru testări s-au luat în lucru preparate semisolide pentru uz topic cu *Amygdali Oleum* în concentraţie 5%, comparativ cu Cicatrizinul® ca preparat semisolid de referinţă. Valorile proliferării celulare au crescut direct proporţional cu timpul de incubare la ambele probe. La concentraţii de 25 mg/mL şi 50 mg/mL, gradul de proliferare celulară a fost mai mare decât la concentraţia de 10

mg/mL. S-au evidențiat valori ale proliferării fibroblastelor umane comparabile cu martorul atât la gelul cu migdale dulci cât şi la Cicatrizin®.

Imaginile de microscopie electronică au arătat că probele nu produc modificări morfologice asupra fibroblastelor umane din cultură. După 72 de ore de incubare celulele şi-au menținut fenotipul şi caracteristicile specifice fibroblastelor normale.

Keywords: gel with *Amygdali Oleum* var. dulcis, cell cultures, cellular proliferation

Introduction

The source of the separated fibroblasts is an essential factor in successfully obtaining a cell culture. Generally it is recommended to sample biological products from young people, the cellular viability depending on the donor's age.

In general, a primary culture is obtained through different procedures, depending on the consistency of the tissue (fluid or solid). In the case of solid tissues, they have to be first dissected into small pieces.

In the explant technique these pieces are placed in culture plates and incubated on adapted growth media in order to encourage cells to migrate out of the tissue and adhere to the surface of the culture dish. After the elimination of the tissular fragments, the cell cultivation may begin.

The fibroblasts are cultivated on adherent surfaces and unistratified cultures are obtained. The cell cultivation needs the presence of fetal bovine serum which contains mithogenic agents with a critical role in the regulation of cellular proliferation. These agents induce the expression of C-jun proto/onchogene and of other members of this gene family collectively named IER (immediate early response). The c-jun nuclear fetoprotein, a major component of the transcription activator complex AP-1, promotes the transcription of many necessary genes for the cellular cycle. The selective inhibition of the expression of the c-jun gene prevents the cells from apoptosis, the programmed cellular death [4, 5].

Various types of cellular lineages are available on the market. Because of one or more genetic mutations that alter their proliferative characteristics, these cells are immortal, multiplying indefinitely. They are obtained by modifying normal cells using UV radiation, chemical agents (tumor promoters) and biological agents (viruses) and are characterized by the loss of contact inhibition and life limitation, morphological alterations, chromosomal anomalies and the capacity to grow on gelose [1, 2, 3, 8, 9].

Materials and methods

In our research we tested the proliferative action on cultured cells of a semisolid preparation for topical use with sweet almonds oil (5% in a

gel basis). The hidrosoluble basis contained: carbopol 980, triethanolamine, propilenglychol, alcohol and water.

The sweet almond oil is extracted by cold pressing (or other mechanical procedures) from the cotyledons of the *Amygdalus communis* var. dulcis. It contains glycerides of the oleic, linoleic, palmitic, stearic fatty acids, fitosterols and tocoferols [6].

Amygdali Oleum is used as a vehicle for oily injectable solutions, as an ointment basis in dermatological and cosmetic products with emollient effect [6, 7].

Cell proliferation

The semisolid preparations with 5% *Amygdali Oleum*, as well as Cicatrizin® (the reference gel) were dissolved in culture medium to final concentrations of 10 mg/mL, 25 mg/mL and 50 mg/mL.

The cellular proliferation rate was determined spectrophotometrically, using the MTT test (3-(4,5-dimethylthiazolium-2-yl)-2,5-diphenyltetrazol bromide). The fibroblasts were cultured on culture plates with 96 wells, at a density of 52 725 cells/mL. After 24 hours of incubation at 37^0C the tested substances were added. After 24, 48 and 72 hours, the culture medium was replaced by a solution of 50 μL MTT dissolved in culture medium and then the cells were incubated for 3 hours. The formazan crystals formed in viable cells were dissolved in iso-propanol, and the absorbance was measured at 570 nm using a spectrophotometer UV-VIS (Jasco V-650, Japan). The proliferation rate was calculated by comparison with the control culture (cells cultured on plastic), considered 100% (proliferation rate).

Cellular morphology

The fibroblast morphology was evaluated by optic microscopy.

The human cells were cultivated on culture dishes with 24 wells and incubated at 37^0C, for 24 hours, and then the tested substances were added to the cellular culture.

The fibroblasts cultivated for 72 hours in the presence of the tested substances were then washed with phosphate- buffered saline (PBS), fixed with methanol, stained with Giemsa and photographed using a contrast phase microscope (Nikon, Japan).

The determinations were performed in comparison with the human fibroblasts cultivated in the absence of the tested substances.

Results and discussion

1. Cell proliferation

The MTT method is based on the reduction of the tetrazol salt to the insoluble, blue, formazan crystals by the mitochondrial dehydrogenases

that use NADH or NADPH as coenzymes. In most cases, the MTT test is used as an indicator of cellular proliferation, because the conversion reaction of formazan salt only takes place in the viable cells and varies proportionally with the number of cells (in the linear domain of the method).

The variation of viability of cultivated fibroblasts in the presence of the tested substances, at various concentrations and at different cultivation moments, was measured spectrophotometricaly.

Over the duration of the test, the results for sweet almond gel, as well as Cicatrizin®, showed smaller values than for the control sample.

In the case of the sweet almond gel we observed 80% proliferation at 10 mg/mL concentration (Fig. 1), 80% and 85% for 25 mg/mL (Fig. 2) and 50 mg/mL (Fig. 3) respectively.

The Cicatrizin®, ointment presented a 90% proliferation rate at 10 mg/mL (Fig. 4), 95% at 25 mg/mL (Fig. 5) and 100 % at 50 mg/mL (Fig. 6), similar to the control sample (Fig. 7).

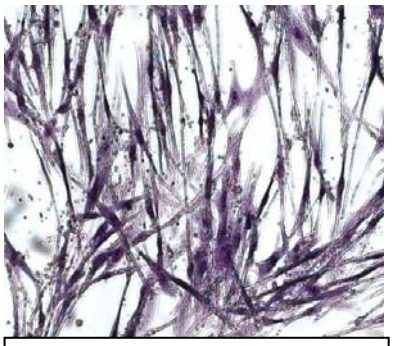

Fig. 1. Cellular proliferation for the sweet almond gel 10 mg/mL

Fig. 2. Cellular proliferation for the sweet almond gel 25 mg/mL

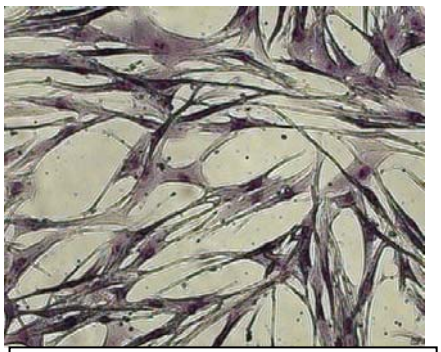

Fig. 3. Cellular proliferation for the sweet almond gel 50 mg/mL

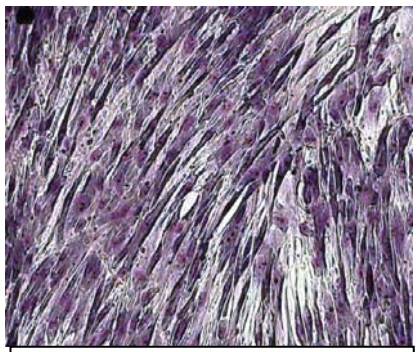

Fig. 4. Cellular proliferation for Cicatrizin® 10 mg/mL

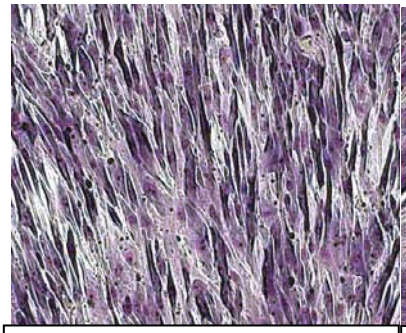

Fig. 5. Cellular proliferation for Cicatrizin® 25 mg/mL

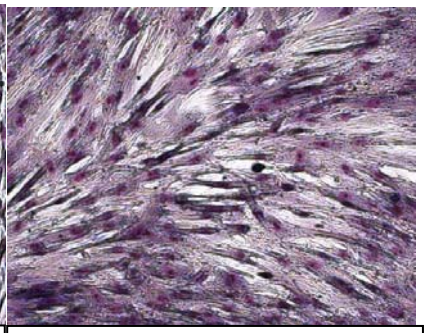

Fig. 6. Cellular proliferation for Cicatrizin® 50 mg/mL

Cellular morphology

Under microscopic examination the cells presented a normal phenotype, similar to the control (Fig .7), in the presence of both sweet almond gel (Fig. 1,2,3) and of Cicatrizin® (Fig. 4,5,6).

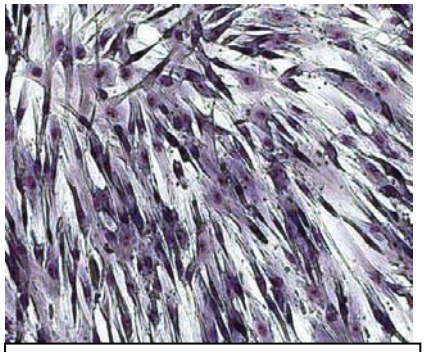

Fig. 7. Cellular proliferation for the control

After 72 hours of incubation, both the control and the cells cultivated in the presence of the tested substances maintained the phenotype and the characteristics of normal fibroblasts. They presented an elongated morphology, with eucromatic nuclei, 1-3 nucleoli and fine cytoplasmic extensions.

Conclusions

The results show that in the presence of 5% sweet almond gel and of Cicatrizin®, the cultured fibroblasts proliferation rate varied with the tested substance, with its concentration, and the cultivation time.

The proliferation rate values increased in direct proportion with the cultivation time. At the concentrations of 25 mg/mL and 50 mg/mL, the cellular proliferation rate was higher than for the 10 mg/mL concentration.

The microscopy images showed that the tested samples did not induce morphological modifications in the cultured human fibroblasts; the cells present a normal phenotype, similar to the control, for both tested substances.

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