SPELEOTHERAPY EFFECTS ON WISTAR RATS REFLECTED BY PULMONARY AND DERMAL FIBROBLASTS CULTURES

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Abstract: Speleotherapy – a special kind of climatotherapy, uses the certain conditions of caves and salt mines to cure several diseases, especially respiratory and skin diseases. Atmospheric dust could cause allergic reactions or asthmatic attacks.The cave air is very low on dust. This fact reduces any kind of irritation. In this way, the symptoms of the diseases are reduced or eliminated completely, while the patient is in the cave. But that does not explain how it should have a longer lasting effect. Curing asthma involves spending 2-3 hours a day underground in subterranean caves or salt mines over a 1-2 month period. An old study describes a speleotherapy course, which was 4 hours a day for 6-8 weeks, with 100 COPD (Chronic Obstructive Pulmonary Disease) and asthma patients and reported improvement that lasted 6 months to 7 years (Skulimowski, 1965).
Our objectives were to explore the effects of speleotherapy on cellular morphology and physiology of pulmonary and dermal fibroblasts obtained from tissues of Wistar rats, in normal and Ovalbumin challenged, "asthmatic" conditions. 60 Wistar rats of 75-100 g weight were divided in two lots: control and ovalbumin challenged animals. Ten animals of each lot were send to Cacica, Turda and Dej Salt Mine for 14 days and maintained in the salt mine medium, as in speleotherapy treatment. Pulmonary and dermal fibroblasts cultures were prepared from Wistar rat lung and respectively dermal tissue.
Trying to identify the biological mechanisms of speleotherapy, our experimental design was made for cell morphology, physiology and biochemical evaluation of cells in cultures obtained from animals that were treated by speleotherapy. The complex picture of results was analysed and explained through biological mechanisms comparing to the control cell cultures obtained from healthy, untreated Wistar rats. In this article we describe the supposed biological mechanisms that explain the protective effects of speleotherapy.
Conclusion: Speleotherapy induces changes on the morphology and protein expression of pulmonary and dermal fibroblasts *in vitro*, and these changes - by comparing with ovalbumin sensitised animals, supports the beneficial effects of speleotherapy.

1. Introduction

Asthma is a disorder characterized by chronic inflammation of the airways, airways hyper-responsiveness, and changes in airway architecture, termed remodeling. The cells responsible for maintenance of lung structure are the parenchymal cells of the lung, including epithelial cells, mesenchymal cells, and endothelial cells. Recent studies have suggested that the function of epithelial cells, smooth muscle cells, and fibroblasts cultured from lungs of individuals with asthma differs from the function of cells similarly cultured from individuals without asthma. These functional differences, particularly as they relate to repair and remodeling, could contribute to airway structural alterations (Sugiura *et al.*, 2007). Bronchial asthma affects up to 10% of the developed countries population, his prevalence increasing in all world.

Therapy with bronchodilators, corticosteroids, leukotriene inhibitors, mastoid cells stabilizers and recent with IgE receptor antagonists have been shown an improvement of asthma symptoms.

To solve the existing problems in allergy, pulmonology and medical recovery field and for use of natural therapeutic factors in patient treatement with different pathologies, international scientific community appealed to specialists, medical, ecological and social programs.

The new scientific and practical directions in therapy of the most severe allergic deseases - bronchial asthma - use underground medium of salt mines and caves. This therapy method was named speleotharapy from greece „spelaion"- cave, gap and „therapy"- treatment. Today the speleotherapy is regognized as therapy in underground of salt mines and caves with natural theraoeutic factors for many deseases (Iu.Simionca and al.,2005, 2008).

Primary cell cultures can readily be obtained from human and animal skin using the explant method or trypsynisation. Full thickness skin, also called the integument, is a composite of three tissues (epidermis, dermis and subcutaneous tissue), none of which constitutes a homogenous entity. Epidermis normally is composed of keratinocytes, which represent the largest population numerically, and lesser numbers of melanocytes, Langerhans'cells, and occasional cells of the lympho-reticular system, which are, however, transient members of the community.

Although the bulk of the dermis is noncellular (collagen and ground substance), within this compartment is also a variety of cell types, including fibroblasts, histicytes, mast cells, macrophages, lymphocytes and Schwann cells, endothelial cells of blood vessels and lymphatics, striated muscle cells of erector pili muscles, and smooth muscle of blood vessels.

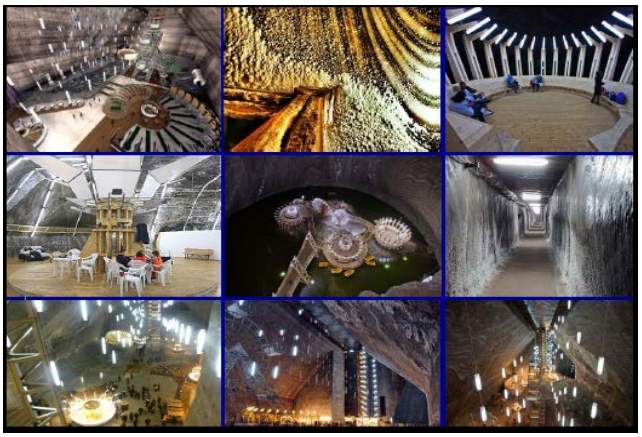


Figure 1. Turda Salt Mine

The subcutaneous tissue includes most of the dermal cell types and fat cells as well (Flaxman, 1974).

The current study was designed to investigate the influence of salt mine medium from Cacica, Turda and Dej Salt Mines upon the cell morphology and electrophoretic expression of pulmonary and dermal fibroblasts *in vitro* obtained from Wistar rats tissues, in normal and Ovalbumin challenged "asthmatic" conditions.

Fibroblasts were cultured from lung and dermal parenchyma of control, ovalbumin-sensitized, and speleotherapy treated rats after ovalbumin-sensitization. Fibroblasts shape in culture can vary in accordance with the substrate, which on they is growing, and the space they have for movement.

Using pulmonary and dermal fibroblasts cultures to verify the therapeutic properties of saline mines medium, described as speleotherapy, represents an innovative and scientific new way to establish the medical methodology of preventing, treating and recovery of patients with various pulmonary problems.

Speleotherapy presents a great scientific interest and is a future direction in health and environmental area.

2. Geography and geology

One of the perspective salt mines used in medical and balenoclimatic tourism purpose from Romania is Turda Salt Mine.

TURDA SALT MINE is one of the historical monuments of Romania, from Cluj and a touristic attraction at national and international level especially for Bai Sarate Turda, Durgau salted lakes and the ruins of Potaissa roman castrum where was stationed the Vth Macedonica Legion 2000 years ago.

The exploitation of salt from Turda in current microdepression of Baile Sarate has a special interest during the roman occupation in Dacia. The first documentary of mine attestation dating from XII century when avid rocks, minerals and fossils collector - Joanne Fridvaldscky says- „is so famous that has no equal in all eastern".

With Saraturile Turzii was declared natural reserve with national interest and became a historycal museum of salt mining.

Turda Salt Mine joined to touristic circuit in 1992 (Ov. Mera si al., 2010) and benefit from EU funding under PHARE CES Programme 2005 through „Improving the attractiveness of the tourist potential of the balneray resort Lacurile Sãrate-Zona Durgãu-Valea Sãratã and Turda Salt Mine" project; modernization works of Turda Salt Mine has start in 2008 and have lasted two years.

Turda Salt Mine has legally all prerequisites, for therapeutic use: mines with furhished rooms, tailored for both tourists and sick persons, including disabled persons, mines rooms are large space, isolated rooms; no exploition activities; in Terezia Mine there are a salin lake adapted for recereation.

Official opening of modernized Turda Salt Mine took place on 22 january 2010.

SALT MINE CACICA - it is situated in the locality with the same name, in the N-E part of the Romania, at 42 km W from Suceava Town and the 17 km N from Gura Humorului. The air strongly ozonized, the purity and beauty of nature, make from this place an attractive destination in any season, both for rest, pleasure and the treatment of respiratory disorders.

The entrance into the salt mine is made on fir tree stairs that are over 200 years old, mineralized by the salty water that penetrated the wood. The work by chisel gab and sledge hammer of the miners that ones worked here left real works of art, that bear the seal of the talent access stairs cut in the salt massif, vaulted ceilings or huge galleries. The real measure of the craftsmanship of those who dug the salt with the hammer is given by the small church built in salt at a depth of 27 metres and the dance hall located at a depth of 37 metres.

This underground Catholic chapel sanctified in 1800 has been gathering all the inhabitants, for the last two centuries, on the feast of Sf. Varvara protector saint of the miners.

OCNA DEJ SALT MINE is located in Romania, in the iddle of the Transylvanian Basin 3 km from the city of Dej and 60 km from Cluj-Napoca. Importance of salt in the development of human civilization and the exceptional quality of the salt deposit made the salt to be exploited since antiquity in Ocna Dej.

The first statement concerning the Ocna Dej salt exploitation dating from Roman times can be observed today in the form of excavation remains clogged. Roman has operated the mine until XII–XIII centuries when they consider the current perimeter begins Ocna Dej salt mining plant.

Today, Ocna Dej salt mine is part of National Salt Company SA and its main activity is extraction, preparation and marketing of gemstones salt.

The Ocna Dej salt mine is characterised by: temperature: 12.4–14.5 ^{0}C, pressure: 1,018–1,020 hPa, humidity: 65–71%, the presence of saline aerosols, lighting artificial and own ventilation system. A higher concentration of NaCl is ensured by continuous operation of the mine.

These environmental conditions provided by the Ocna Dej salt mine led researchers to undertake studies on evaluating the possibility of using this mine, not only for salt extraction, but also for the development of the radon therapy and speleotherapy in Romania (Calin M.R.. and Calin M.A., 2010)

3. Methods

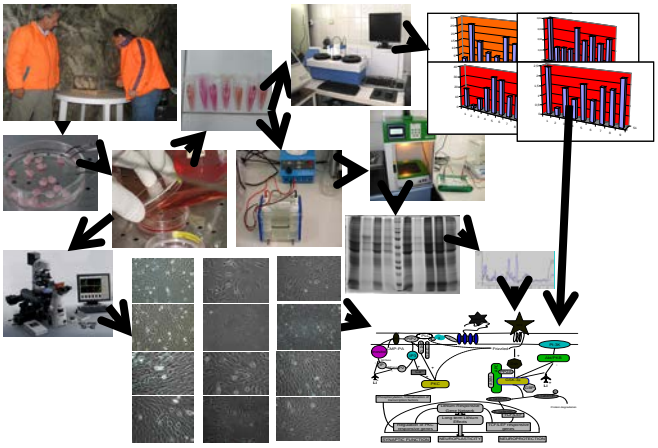


Fig.2 Experimental Design

Materials: Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na_2HPO_4 x12 H_2O 8mM + KH_2PO_4 1,4mM); HAM-F12 culture medium (Sigma); penicillin 100 U/ml, streptomycin 100µg/ml; neomycin 50µg/ml, fetal bovine serum (Sigma).

Rat Wistar Model of Allergic Asthma

Wistar rats of 75-100g weights were sensitized to Ovalbumin by i.m. injections.

Primary pulmonary fibroblasts culture

After anaesthesia with chloroform, rats were killed. The thorax was opened and then the lungs were removed en bloc in a laminar flow hood using sterile technique and put into ice-cold sterile Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na_2HPO_4 x12 H_2O 8mM + KH_2PO_4 1,4mM). 1mm tissue pieces were suspended in 0.125% trypsin and 0.001% DNase and repeatedly stirred for 6 minutes and centrifuged at 1000g. The pellet was resuspended in HAM-F12 medium with 4500mg/l glucose, 25 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µg/ml neomycin and 10% fetal bovine serum (Sugiura *et al*, 2007; Foster *et al*, 1990; Nunez *et al*, 1995).

Primary dermal fibroblasts culture

After anaesthesia with chloroform, rats were killed. The thorax was opened and then the lungs were removed en bloc in a laminar flow hood using sterile technique and put into ice-cold sterile Phosphate Buffer Solution (PBS: NaCl 0,13M + KCl 2,6mM + Na_2HPO_4 x12 H_2O 8mM + KH_2PO_4 1,4mM). 1mm tissue pieces were suspended in 0.125% trypsin and 0.001% DNase and repeatedly stirred for 6 minutes and centrifuged at 1000g. The pellet was resuspended in HAM-F12 medium with 4500mg/l glucose, 25 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin and 50 µg/ml neomycin and 10% fetal bovine serum (Sugiura *et al*, 2007; Foster *et al*, 1990; Nunez *et al*, 1995).

Phase Contrast Microscopy

Phase contrast microscopy, first described in 1934 by Dutch physicist Frits Zernike, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles).

SDS-PAGE Electrophoresis

The proteins electrophoresis from the total homogenate has as the purpose to establish the changes, which are revealed at the proteic level of fibroblasts cultures obtained from rats held on saline mine medium for the speleotherapy.

The proteins electrophoresis in gel of polyacrylamide was done in the denaturated conditions in the conformity with the techniques described by Laemmli (1979). The cultures have been washed with PBS, curetted from the culture plate and lyzed in buffer containing 0,5M Tris-HCl, pH 6,8 + 0,05% BPB + 10% glycerol + SDS 10%.

4. Results

4.1. Speleotherapy results on dermal fibroblasts

Control skin cells culture of 7 days has a heterogenic aspect with a high pre-confluence level. The cell division is to a high level and the cell morphology shows a typical microscopic view, described in the specific literature (fig.3). There are two types of cells: epithelial and fibroblastic.

Skin cells cultures of 7 days obtained from Ovalbumin sensitized rats presents many morphological changes from the control skin cell culture, being observed an sensible number reducing of dermal fibroblasts in culture, the diminished cellular dividing frequency and an accentuated cellular morphopathology of the cells in culture. After 7 days of culturing, the pre-confluence level is much lower than in the control case.

Skin cells cultures of 7 days obtained from Ovalbumin sensitized rats and treated by speleotherapy in Cacica Salt Mine shows an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged rats. By phase contrast microscopy, it is possible to observe a rising of the cells number.

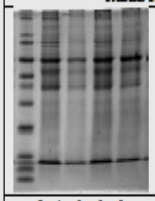
Skin cells cultures of 7 days obtained from Ovalbumin sensitized and treated by speleotherapy in Dej Salt Mine shows also an improvement of the morphological parameters of the cells comparative with the cultures obtained from Ovalbumin-challenged rats. It is observed the rising of the cell population density and that of cell viability.

Skin cells cultures were homogenized with Laemmli buffer pH 6,8, and the proteins of the obtained homogenate were separated by 10 % SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure.

Samples of 10µl were loaded into *wells* in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20,1; 14,2 and 6,5 KDa

Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250, that allowed visualization of the separated proteins. After staining, different proteins appeared as distinct bands within the gel (Towbin *et al.*, 1979).

Analysis with GeneTools version 4 software from SynGene of each track of the electrophoresis, allowed us to compare the profiles of the total proteins expression.

TABLE 1: SDS polyacrylamide gel electrophoresis of the dermal cells cultures	
	Samples
	1- 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine
	2- 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine
	3- 7 days skin cells culture from Ovalbumin-sensitised rats
	4- 7 days Control skin cells culture
5 4 3 2 1	5- Sigma molecular markers

Track 4 7 days Control skin cells culture

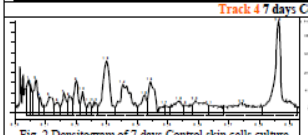


Fig. 2 Densitogram of 7 days Control skin cells culture

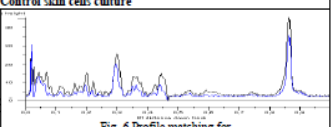


Fig. 6 Profile matching for CONTROL ( ) – OVALBIMN ( )

Track 3 9 days skin cells culture from Ovalbumin-sensitised rats

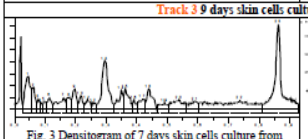


Fig. 3 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats

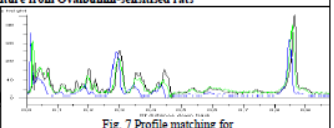


Fig. 7 Profile matching for CONTROL ( ) - DEJ ( ) - CACICA( )

Track 2 9 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

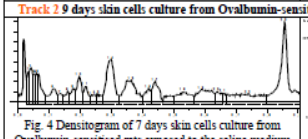


Fig. 4 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine

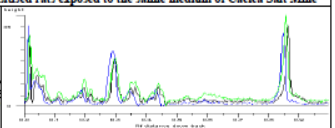


Fig. 8 Profile matching for OVALBUMIN ( ) - DEJ ( ) - CACICA( )

Track 1 9 days dermal cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

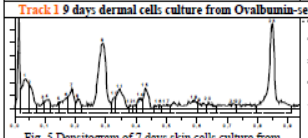


Fig. 5 Densitogram of 7 days skin cells culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine

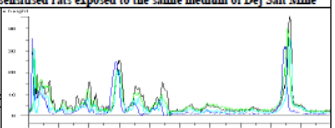
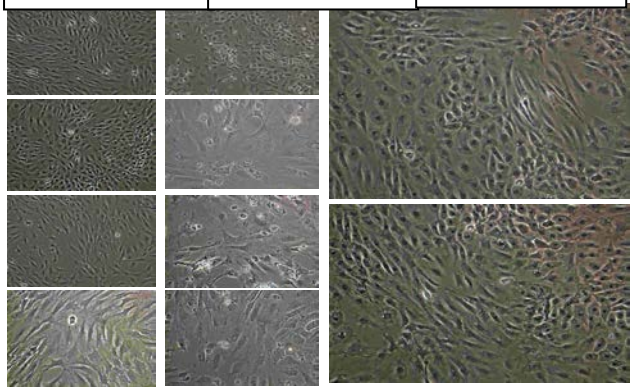


Fig. 9 Profile matching for CONTROL ( ) OVALBUMIN ( ) - DEJ ( ) - CACICA( )

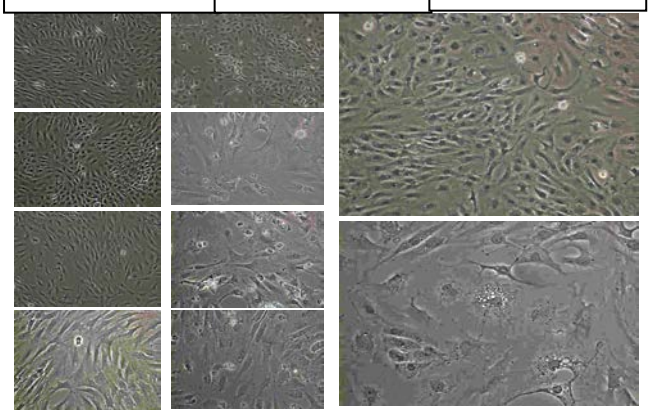
TABLE 2 Protein expression analysis of the skin cells cultures

Peak Nr.	Peak weights molecular limits (KDa)	CONTROL Quantity (µg/10µl)	OVALBUMIN Quantity (µg/10µl)	CACICA Quantity (µg/10µl)	DEJ Quantity (µg/10µl)
1	225 – 240	3,80	5,61	2,60	5,56
2	220 – 225	1,81	2,19	3,58	2,19
3	210 – 220	4,78	0,92	2,07	0,92
4	200 – 210	1,66	0,37	2,04	1,25
5	190 – 200	3,55	0,80	3,86	0,80
6	160 – 190	1,61	1,35	3,39	1,86
7	140 – 160	2,81	2,73	1,12	2,50
8	120– 140	2,53	1,59	1,56	1,13
9	105 – 120	4,73	2,61	2,00	20,03
10	100 –105	2,75	1,45	4,28	1,02
11	90 – 100	1,32	1,36	2,45	3,77
12	63 – 90	1,61	0,90	1,21	0,29
13	55 – 63	13,14	9,95	1,36	0,34
14	42 –55	11,32	2,75	12,25	1,29
15	40 – 42	3,48	3,20	9,62	2,72
16	37 – 40	8,12	0,92	2,72	0,56
17	35 – 37	1,01	1,76	6,35	0,72
18	34 – 35	3,70	3,65	4,73	3,22
19	32 – 34	1,96	1,41	6,55	1,08
20	30 – 32	4,89	3,30	2,28	0,87
21	23 – 30	3,19	2,08	0,86	1,62
22	19 – 23	8,00	10,27	8,11	1,78
23	6 – 19	24,72	18,30	23,20	30,22
TOTAL amount of proteins in 10 µl of sample:		116,5	79,45	108,19	85,74

HEALTHY RATS	"ASTHAMTIC" RATS	CACICA



HEALTHY RATS	"ASTHAMTIC" RATS	DEJ



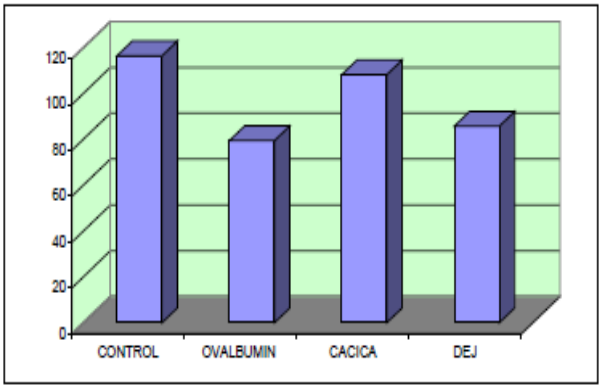


Fig.10 TOTAL amount of proteins in 10 µl of sample

Fig. 3 Experimental results for dermal fibroblasts cultures

4.2. Speleotherapy results on pulmonary fibroblasts

Control pulmonary fibroblasts culture of 9 days has a homogenic aspect with a high pre-confluence level. The cell division is to a high level and the cell morphology shows a typical microscopic view, described in the specific literature.

Pulmonary fibroblasts cultures of 9 days obtained from Ovalbumin sensitized rats presents many morphological changes from the control pulmonary fibroblasts culture, being observed an sensible number reducing of pulmonary fibroblasts in culture, the diminished cellular dividing frequency and an accentuated cellular morphopathology of the cells in culture. After 9 days of culturing, the pre-confluence level is much lower than in the control case.

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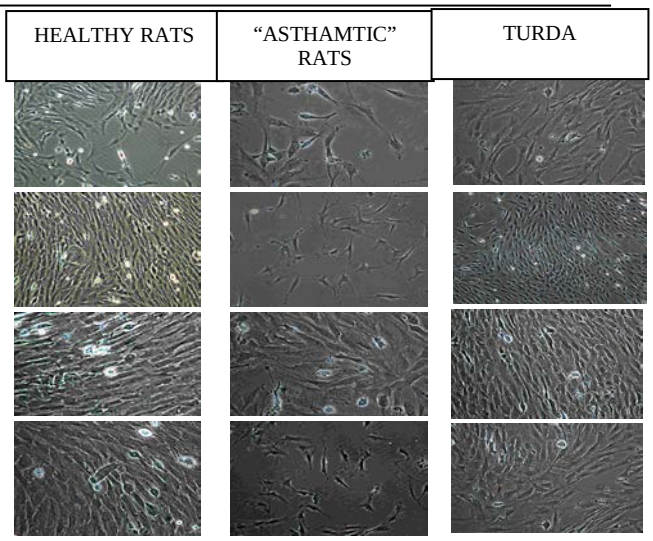
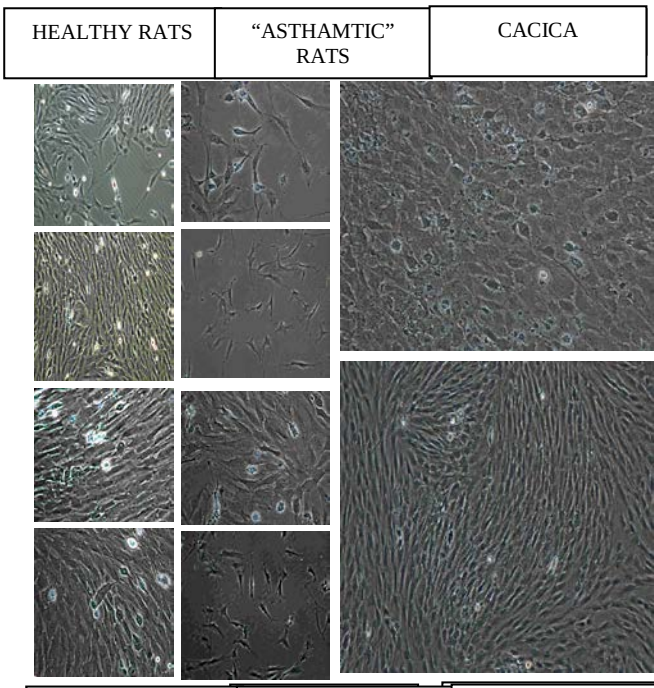
Pulmonary fibroblasts were homogenized with Laemmli buffer pH 6,8, and the proteins of the obtained homogenate were separated by 10 % SDS polyacrylamide gel electrophoresis that maintains polypeptides in a denatured state once they have been treated with strong reducing agents to remove secondary and tertiary structure.

Samples of 10µl were loaded into *wells* in the gel. One lane was reserved for Sigma molecular markers mixture of 205; 116; 97; 66; 55; 45; 36; 29; 24; 20; 14,2 and 6,5 KDa

Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 that allowed visualization of the separated proteins. After staining, different proteins appeared as distinct bands in the gel (Towbin *et al.*, 1979). Analysis with GeneTools version 4 software from SynGene of each track of the electrophoresis, allowed us to compare the profiles of the total proteins expression.





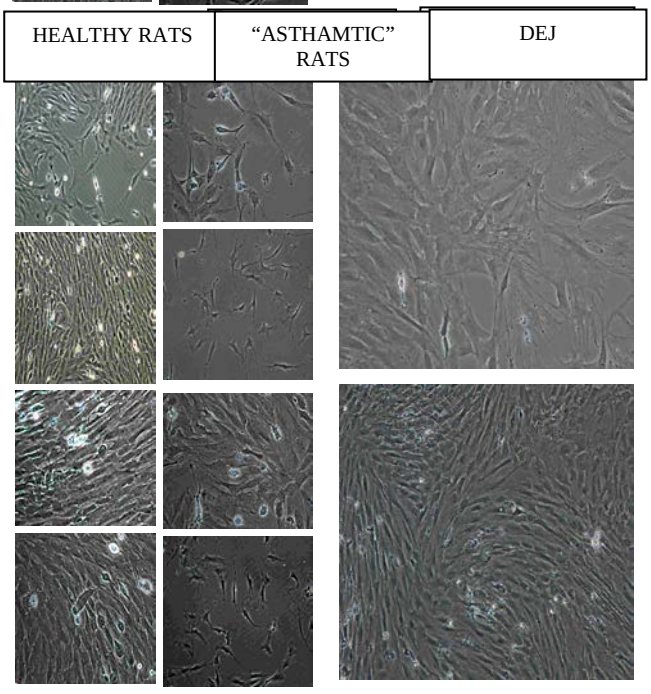
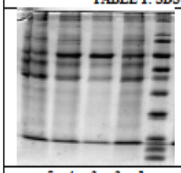
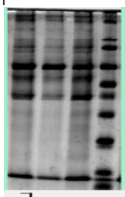


TABLE 1: SDS polyacrylamide gel electrophoresis of the pulmonary fibroblasts cultures



Samples
5- 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Dej Salt Mine
4- 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats exposed to the saline medium of Cacica Salt Mine
3- 9 days pulmonary fibroblasts culture from Ovalbumin-sensitised rats
2- 9 days Control pulmonary fibroblasts culture
1- Sigma molecular markers

Fig. 1 – Electrophoretic profile of pulmonary fibroblasts cultures



PROBA	Cantitatea de proteine per placă
4- cultura de fibroblaste pulmonare expuse mediului din Salina Turda	155 µg
3- cultură de fibroblaste pulmonare obținută de la animale sensibilizate cu ovalbuminã	130 µg
2- cultură de fibroblaste pulmonare martor	160 µg
1- markeri de greutate moleculară (SIGMA)	120 µg / 10 µl

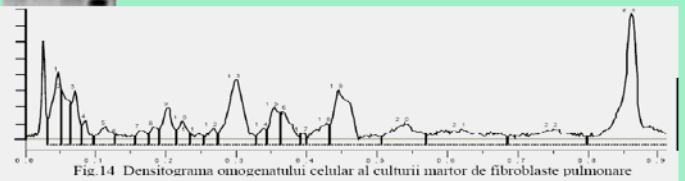


Fig.14 Densitograma omogenatului celular al culturii martor de fibroblaste pulmonare

Fig. 15 Densitograma omogenatului celular al culturii de fibroblaste pulmonare obținută de la animale sensibilizate cu ovalbumina

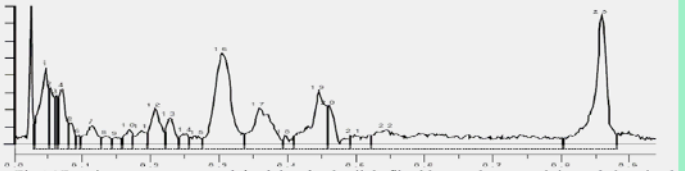


Fig.16 Densitograma omogenatului celular al culturii de fibroblaste pulmonare obținute de la animale sensibilizate și expuse mediului din Salina Turda

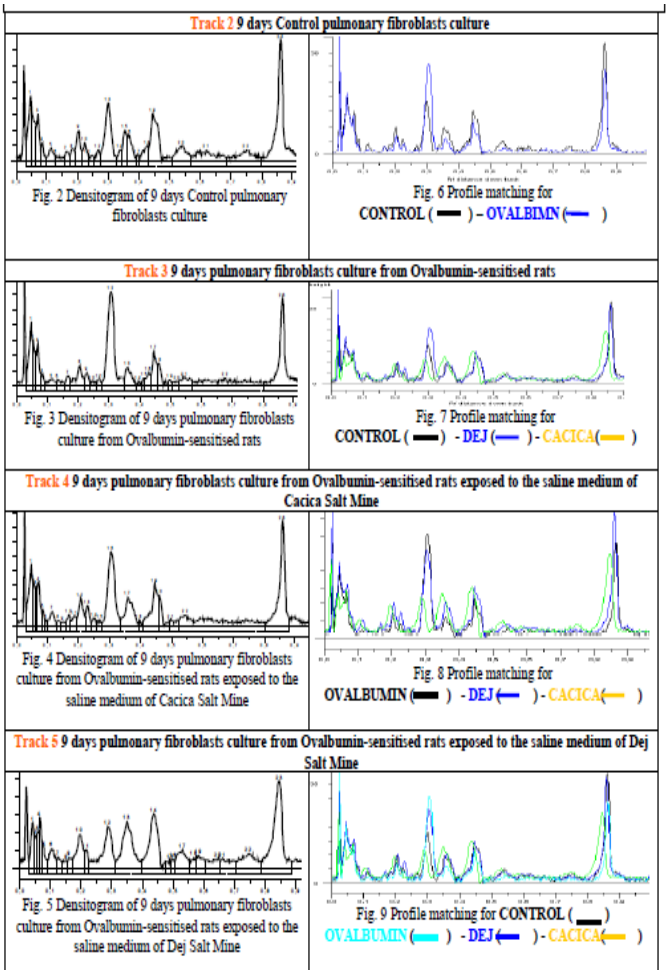


TABLE 2 Protein expression analysis of the pulmonary fibroblasts cultures

Peak Nr.	Peak weights molecular limits (KDa)	CONTROL Quantity (µg/10µl)	OVALBUMIN Quantity (µg/10µl)	CACICA Quantity (µg/10µl)	DEJ Quantity (µg/10µl)
1	225 – 240	5,47	5,18	2,98	6,33
2	220 – 225	3,37	2,35	0,99	2,24
3	210 – 220	2,81	3,08	1,48	1,54
4	200 – 210	1,25	0,56	2,68	3,18
5	190 – 200	1,54	1,23	1,35	1,17
6	160 – 190	0,66	0,65	2,38	0,36
7	140 – 160	0,94	0,90	0,94	2,06
8	120– 140	0,90	2,81	0,70	0,53
9	105 – 120	3,01	1,07	1,00	0,58
10	100 –105	1,58	0,58	4,42	0,98
11	90 – 100	0,59	0,60	1,30	1,34
12	63 – 90	0,94	16,21	8,10	3,38
13	55 – 63	8,77	2,70	10,20	1,96
14	42 –55	0,80	0,34	10,34	0,80
15	40 – 42	2,78	0,39	0,70	0,75
16	37 – 40	2,88	1,38	0,61	14,47
17	35 – 37	0,36	3,11	3,29	6,29
18	34 – 35	2,16	2,16	1,19	0,53
19	32 – 34	8,48	0,44	1,64	7,62
20	30 – 32	3,79	0,55	2,17	2,39
21	23 – 30	4,78	1,86	2,05	1,35
22	19 – 23	4,16	6,64	4,64	12,93
23	6 – 19	18,64	12,62	16,80	15,94
TOTAL amount of proteins in 10 µl of sample:		80,66	67,41	81,95	88,72

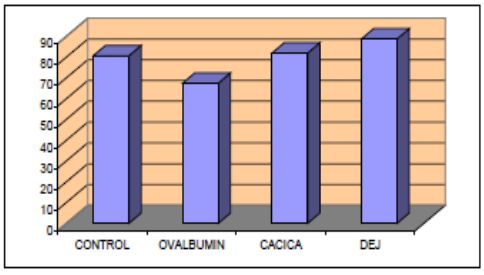
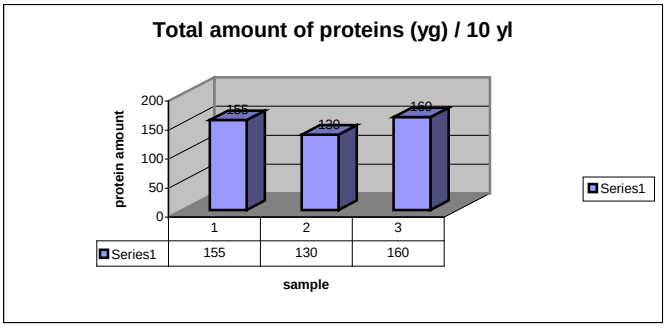


Fig.10 TOTAL amount of proteins in 10 µl of sample



5. Discussion

The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts and epithelial cells obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats.

Compared with control culture, skin cells cultures from Ovalbumin-sensitized rats and Ovalbumin-sensitized treated in Cacica and Dej Salt Mines rats demonstrated the positive role of the saline medium for the sensitized rats.

The current study focused on skin cells, which are believed to play a major role in the organism – environment interaction. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to lesion repair in dermatological problems. Cells cultured from the skin of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura *et al.*, 2007).

The present study evaluated morphological phenotypes related to repair and remodeling in fibroblasts obtained from control Wistar rats and from Ovalbumin-sensitized and -challenged rats, a model of asthma that results in airway hyperresponsiveness and chronic airway remodeling, as other authors had presented.

Compared with control fibroblasts, fibroblasts obtained from lung parenchyma of the "asthmatic" rats and Ovalbumin-sensitized rats treated in Cacica and Dej Salt Mines demonstrated the positive role of the saline medium for the “asthmatic” rats.

The current study focused on fibroblasts, which are believed to be cells that play a major role in the maintenance and remodeling of interstitial connective tissue. In this context, fibroblasts are believed to play a key role in maintaining and altering tissue structure. The ability of fibroblasts to migrate in response to chemotactic stimuli and to proliferate in response to specific growth factors is believed to control their accumulation at sites undergoing tissue repair. The ability of fibroblasts to produce and remodel extracellular matrix is thought to contribute to tissue structural changes. Remodeling of tissues likely involves fibroblast contractile activity.

In summary, the present study supports the concept that phenotypically altered fibroblasts can contribute to airway remodeling in asthma. Fibroblasts cultured from the lungs of chronically OVA-sensitized and -challenged animals demonstrated consistently augmented repair responses for a number of functional assays (Sugiura *et al.*, 2007).

6. Conclusions

- Phase contrast microscopy analyses of primary skin cells cultures reveals an cellular regeneration after animal exposure to saline medium in Cacica and Dej Salt Mines, comparative with the cells morphology of cultures from Ovalbumin sensitised rats.
- The morphological observations are confirmed by the electrophoretic analyses, which demonstrate through rising of the expression of many proteins and of total protein amount that the exposure of Ovalbumin-sensitised animals to the saline medium from Cacica and Dej Salt Mines is reversing the cells morphopathology of skin cells in cultures;
- Wistar rats sensitised with Ovalbumin have a low number fibroblasts in skin cells cultures, with a more sensitive morphopatologic level.
- Phase contrast microscopy analyses of primary fibroblasts cultures reveals an cellular regeneration after animal exposure to saline medium in Cacica and Dej Salt Mines, comparative with the cells morphology of cultures from Ovalbumin sensitized rats.
- The morphological observations are confirmed by the electrophoretic analyses, which demonstrate through rising of the expression of many proteins and of total protein amount that the exposure of Ovalbumin-sensitized animals to the saline medium from Cacica and Dej Salt Mines is reversing the cells morphopathology of pulmonary fibroblasts in cultures;
- Wistar rats sensitized with Ovalbumin have a low number pulmonary fibroblasts output cultures, with a more sensitive morphopatologic level.

Acknowledgments

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References

1. Calin M.R., Calin M.A. – Evaluation of the radon concentration in Ocna Dej salt mine, Romania, J Radioanal Nucl Chem (2010) 286: 169-173, DOI 10.1007/s10967-010-0648-8, URL: http://dx.doi.org/10.1007/s10967-010-0648-8
2. Flaxman A. – cell identification in primary cell cultures from skin, *In vitro*, vol.10, no 1&2, 1974
3. Foster Judith Ann, Celeste B.R., Miller M.F. – Pulmonary Fibroblasts: an in Vitro Model for Emphysema, The Journal of Biological Chemistry, Vol. 265, No. 26, 1990, p. 15544-15549;
4. Horvath Tibor (1986) – Speleotherapy: a special kind of climatotherapy, its role in respiratory rehabilitation, Disability & Rehabilitation, 8:2, 90-92, DOI: 10.3109/03790798609166185, URL: http://dx.doi.org/10.3109/03790798609166185
5. Laemmli U.K. (1979) Cleavage and structural proteins duting the assembly of the head of bacteriophage T_4. *Nature* 227: 680-682.
6. Nunez J.S., Torday J.S. – The Developing Rat Lung Fibroblast and Alveolar Type II Cell Activity Recruit Surfactant Phospholipid Substrate, American Institute of Nutrition, 1995, 1639S-1643S
7. Simionca I., Grudnicki N., Buturuga A., Hoteteu M., Kiss J., Oprina A. – Speleoterapia bolnavilor cu astm bronșic non-sever prin intermediul factorilor terapeutici din salina Slănic Prahova, Editura "George Tofan", Suceava 2009, ISBN 978-973-1862-87-3
8. Sugiura H., Liu X., Duan F., Kawasaki S., Togo S., Kamio K., Wang X.Q., Mao l., Ahn Y., Ertl R.F., Bargar T.W., Berro A., Casale T.B. – Cultured Lung Fibroblasts from Ovalbumin-Challenged "Asthmatic" Mice Differ Functionally from Normal, *Am. J. Respir Cell Mol Biol*, Vol 37, pp 424-430, 2007
9. Towbin H., Staehelin T., Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76: 4350-4354.
10. Skulimowski M. - Treatment of bronchial asthma patients in the chambers of the rock salt mine in Wieliczka, Arch Phys Ther (Leipz). 1965 Nov-Dec;17(6):417-21
11. Karen M. Perrin, Karen S. Dindial - Understanding the Modalities of Complementary and Alternative Asthma Treatments: What Every Health, The International Electronic Journal of Health Education, 2000; 3(1): 6-18