

Spectral and biochemical methods for identification of cellular and tissues malignant changes

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Early detection of malignant transformation is a goal of modern medicine and to this end there is an impressive number of approaches from the scientific field that tries to identify early changes preceding malignant transformation in order to establish a correct diagnosis. This paper aims to combine the optical and biochemical techniques for identifying the changes in membrane dynamics of growth and development of experimental solid tumours. During the purpose has been used experimental Walker 256 carcinoma graft in Wistar rats, followed from day 7 up to day 24 from inoculation of tumour cells. Optical techniques were used fractalometry laser polarization in the preclinical diagnosis of pathological changes and degenerative-dystrophy of experimental tissue, and in terms of biochemical tumour tissue was determined the reaction of lipid peroxidation monitored by the malondialdehyde (MDA), the end product assays of reaction. In addition were followed also the total antioxidants as a response of the endogenous defences systems. The results indicate a rising profile of the processes investigated by the 14th day after tumour graft, following a decrease due to lack of substrate enzymatic reactions, specifically the double links of polyunsaturated fatty acids in the membranes change during tumour development. These data are consistent with the changes of the optical investigated parameters. In conclusion, it can be said that a combination of optical and biochemical methods can provide additional data in order to determine early cellular changes occurring in malignant transformed cells. To track these changes in experimental tumour growth dynamics is useful for determining the optimal timing of oncostatic treatment intervention.

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1. Introduction

The malignant transformation of normal cell is not a rare event, considering that any cell in the normal cycle division has the potential to become malignant. However most people go through life without a cancer, the detection and control of the body are able to stop the cells to be transformed in tumours.

Although malignant tumour cells take birth in normal cells, their morphology is much changed from the cells of origin. Optical microscopy studies have allowed to shape characteristics of cellular abnormalities.

Biological membranes plays a crucial role in all cellular phenomena. The main membranes constituents are lipids and proteins.

One of the major progress made in biological and biochemical studies of biological membranes is that they are not static entities with rigid structures [1,2]. And maintaining the structure of liquid crystal, both lipids and proteins have freedom of movement. Flow can be regarded as disorderly movement of individual carbohydrate chains of molecules double layer. Mobility and rigidity of protein and lipid molecules is favoured by temperature and high degree of unsaturated carbohydrate links and the length of carbohydrate chains. Decreased fluidity can be observed experimentally by lowering the temperature. If a high percentage of fat molecules in the membrane are unsaturated, the melting temperature membrane is lower

than body temperature. In this way a part of the double layer is fluid, and the polar groups of lipid molecules can move freely.

After the malignant transformation, the cytoplasm membrane is the first modified structure [3,4].

Early detection of malignant transformation is a goal of modern medicine and to this end there is an impressive number of approaches from the scientific field that tries to identify early changes preceding malignant transformation in order to establish a correct diagnosis.

This paper aims to combination the optical and biochemical techniques for identifying the changes in membrane dynamics of growth and development of experimental solid tumours.

2. Materials and methods

Tumours obtained in murine models are considered experimental and currently an important tool in the elucidation of malignant mechanisms, no one perfect model for clinical oncology. Transplanted tumours in animals experience are different from human tumours in several respects: the rate of growth much faster, less heterogeneous cell expressed, develop-invasive and infiltration capacity much lower metastasis. A major disadvantage of transplanted tumours is the appearance of

subpopulations with differentiated cell biological behaviour from parental cells.

Despite these many shortcomings, murine tumours transplanted in conjunction with in vitro models (cell culture, tissue and organs) to continue support of the biological basis for various cancer studies.

Walker 256 carcinosarcoma is a standard used tumour in the preclinical screening of anti-substances, as well as in various experimental models of chemo-radio-immunotherapy.

Percentage of holding a subcutaneous tumour grafts is 80-90%. In Wistar rats the metastasis are rare, and the invasion of loco-regional nodes occurs in the terminal phase of tumour growth when tumour necrosis phenomena are emphasized.

Researches were conducted on male Wistar rats with average weight of 110 g, clinically healthy, from the animal house of the Institute of Oncology from Bucharest. Tumoral implant was performed with a suspension of cells, about 1×10^7 cells in 1.0 ml saline buffer subcutaneous injection in the right flank. At 7 days after the cells inoculation the first clinical manifestations appears, tumour was tangible after 14 days [17-20].

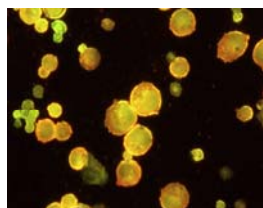


Fig. 1. Walker cells - with acridine orange colour; x20 objective. Walker cells DNA-labelled with PI - fluorescent red; x20 objective.

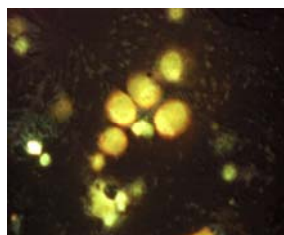


Fig. 2. Walker cells - with acridine orange colour; x20 objective. DNA fluorescent green - yellow, red RNA fluorescence flame red or claret

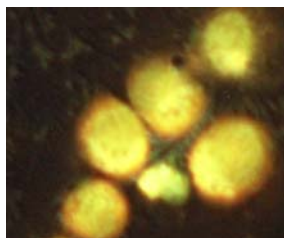


Fig. 3. Walker cells - with acridine orange colour: DNA fluorescent green - yellow, red RNA fluorescence flame red or claret.

Tumoral volume for each tumour is calculated in part by the relationship:

$$V_{\text{tumoral}} = a \times b^2 \times 0.52 \quad (1)$$

where: a = large diameter of tumour (mm); b = diameter of small tumour (mm); tegument thickness = 0.52 (mm).

2.1 Preparation of biological samples

Blood was collected by cardiac puncture (without anticoagulant), centrifuge at 3000 rpm for 15 minutes. The serum was used for analysis.

After taking each sample tumoral tissue and liver were obtained homogeneous tissues grown by grinding with saline (at 1 g of tissue were added 10 ml saline). After centrifugation at 3000 rpm was used for analysis serum.

2.2 Biochemical assays

Determination of lipid peroxides was performed in serum, tissue and tumoral liver tissue [5,6,15].

Determination of thiol groups was performed in tissue and tumoral liver tissue [10].

2.3 Optical setup and experiment

For the conduction of such investigations a new installation was created which optical scheme is shown in Fig. 4.

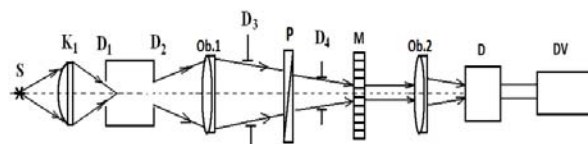


Fig. 4. Optical scheme of the installation.

Here: S – a stabilized source of radiation; K1 – a lighted condenser; D1 and D2 – an entrance and exit slot of the monochromator MUM; Ob.1 and Ob.2 – objective glasses; D3 and D4 – diaphragms; P – a polarizer, Nicol prism; M – an investigated sample; D – radiation detector: в in the area $\lambda = 330 \div 600$ nm photomultiplier, in the area $\lambda = 600 \div 750$ nm the silicon photodiode FD-24K; DV – digital voltmeter.

3. Results and discussion

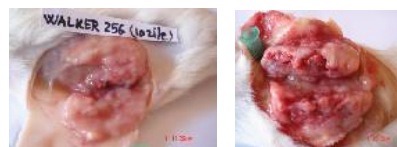


Fig. 5. Walker carcinoma 256.

Tumour is well vascularise with dilated vessels near the numerous outbreaks of necrosis. Lipid Peroxidation is

a classic example of the radicals mechanism chained reaction.(16) Although the auto oxidation or lipids peroxidation under the action of reactive oxygen species is a widely accepted phenomenon, mechanisms and consequences of its interpretation is very controversial due to the great diversification [7,8]. This can be explained by the composition of extremely varied quality and quantity of lipids in polyunsaturated fatty acids by different experimental conditions. Lipid peroxides were assessed by measuring the concentration of MDA, the end product of lipid degradation [9,11,13,18,19].

	14 days	17 days	24 days
Ser	6,07µmol/100ml	9,74µmol/100ml	7,83µmol/100ml
Peritumoral tissue	4µmol/100 ml	5,26µmol/100ml	6,19µmol/100ml
Tumoral tissue	7.76µmol/100ml	9,91µmol/100ml	7,93µmol/100ml

Action of active oxygen metabolites in the structural proteins or enzymes cause distortion thereof. In most frequently interactions occur also in thiol groups of proteins which in turn may undergo oxidation or exposure of thiol groups of proteins is essential [10,12]. This explains the increased sensitivity to oxidation of denatured proteins (this property is used as an effective method of identifying content in these groups and to identify secondary and tertiary structure of proteins.

Total albuminic thiol groups were determined by reaction with acid-5.5 'ditio-bis (2-nitrobenzoic) (Elman reagent), DTNB noted.

	14 days	17 days	24 days
Ser	566 µmol /l	215 µmol /l	104 µmol /l
Peritumoral tissue	640 µmol /l	540 µmol /l	218 µmol /l
Tumoral tissue	701 µmol /l	495 µmol /l	344 µmol /l

Oxidative stress may be defined as a dynamic imbalance between the production in excess of reactive oxygen species to the detriment of their capture and neutralization of the endogenous antioxidant systems [14]. When installed, oxidative stress may be reversible and is a production of free radicals in excess, but for a shorter period, or when action is irreversible for a long time, the concentration of free radicals and the higher effects are disastrous for the integrity and functioning of cell.

3.1 The linear dichroism of bio tissues with different types: with a benign tumour and a malignant tumour

In order to investigate thin tissue cuts of the tumoral tissues were produced. Unfortunately, the investigation was possible only on some of those cuts as a great amount of samples turned to be so small that the cutover of the beam exceeded their magnitudes. Despite this, the results of the investigation on different types of tumours (4-5 samples for each type) can be regarded as those that have general character.

The results of the investigation of the tumour tissues showed that the magnitude of the linear dichroism Δ is

insignificant in the whole researched spectral range $\lambda=280\div840\text{nm}$ and specific regularities in its change aren't observed.

The results of the investigation of the malignant tumour tissues in the form of spectral dependence of the magnitude Δ for three samples are showed on the Fig. 6. It is seen that in the area $\lambda<320\text{ nm}$ and at $\lambda>740\div800\text{ nm}$ the linear dichroism is almost lacking; the maximum values Δ fall on $\lambda=410\text{ nm}$ and are in the range of 8 to 22% according to individual peculiarities of a patient [23].

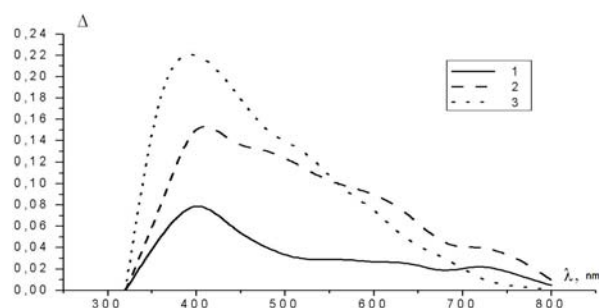


Fig. 6. Spectral dependence of magnitude of the linear dichroism of the prostate gland:
1-sample no. 53; 2-sample no.65; 3-sample no.63.

The corresponding transmission factors are figured out:

$$\tau_0 = \frac{I_{\min}}{I_0}; \quad \tau_{90} = \frac{I_{\max}}{I_0} \quad (2)$$

the magnitude of the linear dichroism:

$$\Delta = \tau_{90} - \tau_0 = \frac{I_{\max} - I_{\min}}{I_0}, \quad (3)$$

the mean factor of the sample transmission:

$$\tau_{\text{cep}} = \frac{I_{\max} + I_{\min}}{2I_0}. \quad (4)$$

3.2 The linear dichroism magnitude change dynamics of the rats bio tissues in the spectral range of $\lambda=330\div750\text{ nm}$

The results of the investigation for each rat on different stages of cancer disease development were recorded in the table which is similar. As not all the samples were good for the investigation (heterogeneity of the beam thickness, insufficient size of the sample and as the result the radiation beam with the diameter of 6 mm went out of these sizes), that's why we managed to obtain only the full set of tables for different stages of the cancer disease development only for three rats. It is evidently that the similar regularity is noticed for all the rats: the linear dichroism grows practically in the investigated spectral

range with the growth of the cancer disease time about to 47÷50 days its decrease is noticed.

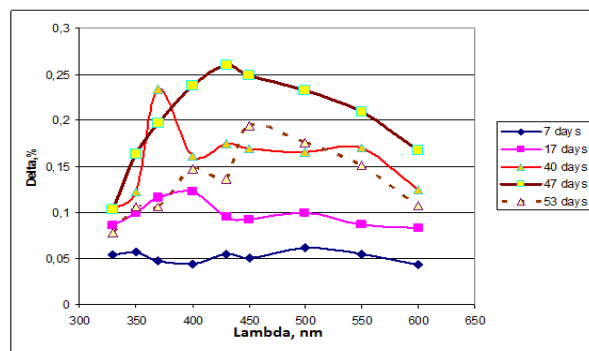


Fig. 7. The spectral magnitude dependence of the linear dichroism which is mean for all the rats: 1) - 7 days after the contamination; 2) - 17 days after the contamination; 3) - 40 days after the contamination; 4) - 47 days after the contamination; 5) - 52 days after the contamination.

4. Conclusions

The following conclusions are made on the results of the investigation:

1. It is shown that in all the cases the linear dichroism appears in bio tissues with the cancer disease the magnitude of which depends on the tumour growth of the cancer process development.

2. The phenomenon of the linear dichroism formation has a selective character: maximum values Δ are observed in the area $\lambda=410\div430$ nm and in the area $\lambda=500\div530$ nm, for the wavelength $\lambda<300$ nm and $\lambda>750$ nm Δ is almost zero or zero.

3. The linear dichroism magnitude depends on the thickness of samples that's why at thicknesses $d=10\div12$ μm when the transmission is 80% and more, it doesn't become apparent at the measurements on the spheric photometer, in this case it is better to conduct the transmission investigation in the direct beam.

4. As the linear dichroism is lacking for healthy tissues, then the obtained results can have diagnostic values with the purpose of detection and estimation of the stage of the cancer disease development [20-22].

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References

- [1] H.S.Basaga, Biochem.Cell Biol. **68**, 989 (1990).
- [2] H. Tkac, L. Bahna, Neoplasma **29**, 497 (1982).
- [3] E. Cross, B. Halliwell, Ann. Int. Med. **107**, 526 (1987).
- [4] J. M.Gutteridge, Chem. Biol. Interact. **91**, 133 (1994).
- [5] T. A.Dix, J. Aikens, Chem. Res. Toxicol. **6**, 2 (1993).
- [6] J. E. Biaglow, J. B. Mitchell, K. Held, Int. J. Rad. Onc. Biol. Phys. **22**, 665 (1992).
- [7] R. G. Cutler, The relationship between radicals formation and life span. Physiology of oxygen radicals, A. E. Taylor and P. Ward eds, Bethesda, 368 (1989).
- [8] M. A. Rodriguez, A. Ruiz-Torres, Mech. Age Develop. **66**, 213 (1992).
- [9] M. Dizdaroglu, Free Rad. Biol. Med. **10**, 225 (1991).
- [10] R. T. Dean, J. V. Hunt, A. J. Grant, Y. Yamamoto, E. Niko, Free Rad. Biol. Med. **11**, 161 (1991).
- [11] J. B. Kirkland, Biochim. Biophys. Acta **319**, 1093 (1991).
- [12] B. Workman, Int. J. Rad. Oncol. Biol. Phys. **22**, 631 (1992).
- [13] K. Haughe, M. Filip, J. L. Witztum, J. Clin. Invest **86**, 1991 (1990).
- [14] G. Cao, R. L. Prior, Clin. Chem. **44**, 1309 (1998).
- [15] R. Kandar, V. Muzakova, A. Cegan, Clin. Chem. Lab. Med. **40**, 1032 (2002).
- [16] H. Esterbauer, R. J. Schaur, H. Zollner, Free Rad. Bio. Med. **11**, 81 (1991).
- [17] L. L. Zwart, J. H. N. Meerman, J. N. M. Commandeur, N. P. E. Vermeulen, Free Rad. Biol. Med. **26**, 202 (1999).
- [18] D. R. Janero, Free Rad. Biol. Med. **9**, 515 (1990).
- [19] M. U. Dianzani, Tumori **75**, 351 (1989).
- [20] M. I. Gruia, R. Olinescu, M. Marinescu, I. Gruia, Rom. J. Comp. Oncol. **4**, 268 (2001).
- [21] O. V. Angelsky, Yu. Y. Tomka, A. G. Ushenko, Ye. G. Ushenko, S. B. Yermolenko, Yu. A. Ushenko, Proc. SPIE, **5972**, 158 (2005).
- [22] O. V. Angelsky, S. B. Yermolenko, O. G. Prydij, A. G. Ushenko, Ye. G. Ushenko, J. Holography Speckle **3**, 27 (2006).
- [23] A. Ushenko, S. Yermolenko, A. Prydij, S. Guminetsky, I. Gruia, O. Toma, K. Vladychenko, Proc. of SPIE **7008**, P.70082C-1 (2008).

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