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Immune state of patients: detection using fluorescent response

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Abstract

An immune state of patient is the first question in medicine. The fluorescent probe offers clinicians a quick, reliable, and reproducible method. The material is presents findings of investigating the possibility of using the original fluorescent probe ABM (an aminoderivative of benzanthrone) for the detection of structural/functional alterations in blood plasma albumin and among immunocompetent cells in patients with different pathologies (non-malignant and malignant). We registered probe ABM spectral parameters in patients lymphocytes, blood plasma, and ABM auto-fluorescence in plasma. The fluorescence intensity of ABM in blood plasma and lymphocyte suspension differed from control values and showed specific differences in patient groups in accordance with immune status of patients. A significant decrease in ABM fluorescence in plasma could be explained, in part, by a diminished binding capacity of the albumin of these patients. The ABM fluorescence in cell suspension and blood plasma was found to correlate with select immunological parameters (CD4+/CD8+ ratious, lymphocyte counts, etc.). There was a strong agreement between changes in ABM spectral parameters and both clinical and pathological estimates of disease severity. Measures of ABM spectral characteristics could potentially be a useful tool to estimate the immune status of patients. Compared to many commonly used diagnostic protocols, the fluorescence based method is sensitive, less expensive and time consuming, technically simple and convenient.

Keywords: Immune state, fluorescent probes, lymphocytes, albumin

Introduction

In different pathologies, damage in the membrane of immune cells and blood plasma albumin involves, as a consequence, alterations in the immune state of patients. It is now widely accepted that the dynamics of these changes, along with certain types of alterations in structures of the immune system, cells, and plasma proteins play a critical role in the maintenance of the immune status of any given organism. The significance of changes in the structural integrity of the immune system cells makes it important for clinicians to receive information on the immunological status of organism via quick, reliable, and reproducible methods. In this regard, fluorescent probes have proven to be an excellent tool for using in such protocols [1-4]. Fluorescent analysis not only has a great potential for helping to comprehend the mechanisms of immunomodulation associated with the induction/progression of pathologies, but might also serve as a very important prognostic indicator of long-term survival among patients with pathologies. This article is based upon findings of the immune state investigation in patients with different pathologies using an original probe ABM (derivative of benzanthrone). Only a brief review of results is given in the article; a more detailed description of all investigated pathologies and patients groups can be found in the book [1] and article [5].

Review

Characteristics of the probe ABM

Spectral characteristics of aminobenzanthrones (e.g., ABM) satisfy all requirements for an ideal tracer (bright fluorescence, high extinction coefficient, photo-, thermo-, and chemical stability) [6,7]. Specifically, it was found out that spectral characteristics of this probe correlate with a number of important parameters of artificial and cellular membranes such as their physicochemical state, microviscosity, proliferating and lipid metabolic activities of cells, distribution of lymphoid subset, etc. [8,9].

Advantages of the probe ABM

Albumin is chosen for examination among myriads of plasma constituents because this protein is practically the only source of ABM binding and subsequent fluorescence in plasma. It is also very important that ABM can be used as a probe that is sensitive to conformation changes in protein: the most prominent changes in fluorescence characteristics occur at the pH values of 3-12 known to cause conformation transitions of proteins [10]. It is significant to note that the probe emission in the red region of spectrum (650 nm) significantly improves analytical sensitivity of the method (as compared with such probes as ANS, K-35, PR, etc.; see **Table 1**) [2,3,11-13].

For example, according to the literature data, the probe ANS possesses a low sensitivity to conformation changes of albumin at pH 5.5-11.5. It was concluded that, because of its fluorescent and binding properties, the probe PR may be considered as a more 'red' long-wave analogue of the probe ANS.

Only fluorescent probes allow detecting the "effective" concentration of albumin in blood plasma ("effective" concentration is

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Table 1. Optical characteristics and spectral parameters of probes binding with blood plasma albumin.

Parameter	ANS	K-35	PR	ABM
Maxima excitation and emission of fluorescence (nm) of probes in complex with albumin	370/470	430/515	525/625	470/650
Stokes shift (nm)	100	83	100	180
Quantum yields of fluorescence in complex with albumin (QY)	0.5-0.9	0.12	0.8	0.08
Constant of binding (x $10^5 M^{-1}$)	5.9	1.8	4.7	1.8
Binding sites number per 1 molecule of albumin (human plasma albumin standard)	2-3	2	2	2-3
Parameter of fluorescence activation: ratio of fluorescence intensity of bound (Fb) with albumin and free (Ff) probe	105	18	34	70

Notes and references:
ANS: (8-Anilinonaphthalene-1-sulfonic acid) [2,3,13]
K-35:N-carboxyphenylimide of 4-dimethylamino-1,8-naphthalenedicarbonic acid [2,3]
PR: Pirrone red [2,3]
ABM: Derivative of 3-aminobenzanthrone [1,5]

equivalent to healthy albumin in blood plasma and reflects its binding and carrier functions). The total albumin concentration is more conservative. The detection of an "effective" concentration of albumin using ABM is technically simple and not so time-consuming in comparison with other probes, as it does not require additional steps of plasma and probe preparation [2,3]. ABM spectral parameters in blood plasma are coupled with alterations in cellular mechanisms of immune regulation in the patients with various pathologies. Measures of ABM fluorescence intensity values for plasma albumin and/or for lymphocytes (total and among different subtypes) could potentially be a useful tool in clinical immunological screenings aimed at estimating the immune state of patients.

Non-malignant diseases [1,5,14,15]

ABM was used to characterize the lymphocytes of patients with several non-malignant diseases, advanced lung tuberculosis, multiple sclerosis, rheumatoid arthritis. The wavelength maxima of these patients did not differ from healthy donors. These groups showed only differences in the fluorescence intensity. The fluorescence intensity and functional activity of cells in tuberculosis patients was found to depend on the nature and dynamics of the tuberculosis process and predominance of the oxidative or productive inflammation phase. The above mentioned parameters also changed according to the phase (exacerbation or remission) and type (remitting or chronic progressive) of multiple sclerosis, and seropositive or seronegative form of rheumatoid arthritis.

Colorectalcancer [1,5,8,9,16,17]

A fluorescent probe ABM was used to characterize lymphocyte membranes and blood plasma albumin from colorectal cancer patients in the context of the hosts' immunological parameters and state of cancer progression. For the study, the following types of patients with colorectal cancer were examined: 1) patients one day before and 10 days after their surgical treatment; 2) patients in the state of worsened disease (Stages IIa, IIIb, and IV); 3) advanced cancer patients. The aim of the study was to evaluate the potential utility of measuring ABM fluorescence parameters as a standard tool in the analysis of host immune status and for a clinical interpretation of alterations in albumin per se and functional activity of lymphocytes in patients. ABM spectral parameters in lymphocytes reflect interrelated properties of the cells: 1) physicochemical state of the outer membrane, 2) membrane microviscosity, 3) proliferative activity, 4) lipid metabolism, and/or 5) phenotypical profile. ABM binds with blood plasma albumin with a high level of selectivity. The probe ABM is very sensitive to all known conformational changes of albumin in the region of pH 3-12. Spectral parameters of ABM binding with plasma albumin reflect albumin "effective" concentration (equivalent of "healthy" albumin in patient's plasma), alterations of albumin globule, and its physical and functional properties, characteristics of binding sites properties. A significant decrease of ABM fluorescence intensity in plasma could be explained by binding capacity/conformational changes of the albumin in these patients. Specific relationship was found out between ABM fluorescence in lymphocytes and characteristics of cells: anisotropy index, binding constant, binding sites number, etc. The lymphocyte distribution among the subsets also differed. Interestingly, the ABM fluorescence intensity in the cell suspension and blood plasma correlate with select immunological parameters (CD4+, CD8+, ratio CD4+/CD8+, CD38+, CD16+, level of immunoglobulines IgA, IgG, IgM, etc.). A decrease in the CD4+/CD8+ ratio mainly depends on an increase in the T-suppressor cells in patients without metastases, whereas it is due to a decrease in the T-helper cell in most patients with a metastatic disease. A surgical treatment affects immunological parameters and appeared to raise functional activity of lymphocytes. The preoperative immune state of patients is important for their survival. Immunosupression increased gradually with the progress of cancer, and capacity of albumin binding reserve and "effective" concentration decreases. These findings suggest that physical (structural) and functional alterations in the cells and plasma of patients were a function of cancer

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stage. In advanced cancer, in contrast to other groups, the absolute number of lymphocytes had a direct, rather than inverse, correlation with ABM fluorescence intensity. A bigger number of lymphocyte, a higher level of T-cell proliferative activity and albumin "effective" concentration have a beneficial effect on overall survival. There was an excellent agreement between changes in spectral characteristics and both clinical and pathological estimates of disease severity. Measurement of ABM fluorescence intensity values in blood plasma and lymphocytes (as a reflection of their functional activity) might be a useful tool in the evaluation of the immune status of patients in clinics, including prognosis, prediction of therapeutic efficacy, and treatment outcomes. The fluorescence-based method is less expensive, not very time-consuming, technically simple, and 100 times more sensitive than standard ones.

Radiation effect [18-21]

Around 6000 inhabitants (20-49 years old) of Latvia took part in clean-up works in Chernobyl in 1986-1991. Most of them were officially documented as recipients of ionizing radiation exposure 0-50 cGy. Examination of these people was performed consistently from 1997 to 2013.

The obtained patterns of ABM spectra suggest that specific and qualitatively different changes of membrane properties are evident in the cells of Chernobyl clean-up workers, similar to those in lymphoid leukemia patients. A correlation was obtained between probe fluorescence intensity, anisotropy, and the ability of cells to produce interferons when induced *in vitro* by Newcastle disease virus or phytohemagglutinin.

It is widely accepted that the dynamics of plasma proteins (e.g., albumin) play a prime role in immune characteristics of humans. Albumin is the only source of ABM fluorescence in human blood plasma (650 nm). The aim of the described study was to determine several aspects of blood plasma albumin alterations in those Chernobyl clean-up workers who had worked within the zone from 1986 till 1991. Most of them were officially documented as recipients of ionizing radiation exposure (1-50 cGy). Various groups of Chernobyl clean-up workers (workers wit no explicit pathologies, those after myocardial infarction, workers with epilepsy paroxysm, and with Type 2 diabetes mellitus) were selected for the study together with corresponding groups of patients having no professional contact with radioactivity. The following parameters were examined: 1) the spectral characteristics of ABM in blood plasma, 2) plasma auto-fluorescence parameters; 3) "effective" and total albumin concentrations in blood plasma ("effective" concentration is equivalent to that of healthy albumin in plasma and reflects its binding and carrier functions). The total albumin concentration is more conservative. It was noted that changes of pH in the range 3-12 strongly affect spectral characteristics of ABM bound with albumin. The spectral characteristics of ABM in Chernobyl clean-up workers plasma result in the splitting of albumin alterations into two stages, an acidic expansion stage (620-630 nm) and N-F transition stage (600-620 nm). These observations might be consistent with the decreased binding of ABM and/or conformational changes of an albumin molecule, significantly differing in the observed groups of patients. The levels of pathological and pharmacological metabolites (fatty acids, antioxidants, plasma levels of lipid peroxidation products, etc.) balance differs in the studied patient groups comparing to controls, and hence their correlation to seizures pathophysiology and their degree. The metabolites caused conformational changes in albumin molecules and shifts in binding parameters are in agreement with the results of albumin auto-fluorescence data and characteristics of ABM binding sites (binding sites count, affinity for probe, and polarity). The results clarify the heterogeneous nature of ABM binding and reveal quantitatively different conformations of albumin in the observed groups of patients. The spectral parameters of ABM in plasma (as in lymphocytes) are coupled with cellular mechanisms of immune regulation in patients. The results also show a strong correlation with select immunological parameters (CD4+: CD8+ ratios, lymphocyte count, etc.) and both clinical and pathological estimates. More pronounced albumin structural/functional alterations were observed in clean-up workers with concomitant diseases. One can state with a certain degree of confidence that the revealed alterations of albumin are dependent on radiation-induced factors. Concomitant diseases reinforce radiation-induced effects in accordance with its manifestation. Therefore it appears likely that external radiation and incorporated radionuclide predominate in alterations of albumin. Measuring ABM characteristics in blood plasma could potentially be a useful tool to estimate the immune state of patients in clinical immunological screenings.

Type 2 diabetes mellitus [20,21]

Alterations of Blood Plasma Albumin in Chernobyl Clean-up Workers with and without Type 2 Diabetes Mellitus. Diabetes mellitus has been one of the most crippling diseases that man has seen; and its the number of people suffering from it has risen dramatically over the past two decades. Currently there are over 150 million diabetics word-wide and this number is likely to increase to 300 million or more by the year 2025. Diabetes mellitus increases the risk of many disorders including cardio-vascular diseases. Understanding the molecular properties of diabetic progression is a big challenge in systems-biology era.

The aim of this study was to determine several aspects of plasma albumin alterations in the group of Chernobyl clean-up workers who have developed diabetes mellitus in comparison with a group workers without the developed diabetes mellitus and patients having no professional contact with radioactivity. Patients in Group 1, with Type 2 diabetes mellitus, have complications associated with diabetes such as polyneuropathy, atherosclerosis, including

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chronic diseases with inflammation. In Group 2, several chronic diseases was noted such as cardiovascular disorders, progressive atherosclerosis with an active inflammation process, leucocytosis, metabolic syndrome, and stroke. Group 2 contains Chernobyl clean-up workers without diabetes mellitus. ABM fluorescent characteristics are dependent on functional activity of CNS and indices of inflammatory processes. The most serious combination of diseases for patients is holisterinemy, progressive cerebral atherosclerosis, epilepsy paroxysms. In all groups, the following parameters were examined: 1) the spectral characteristics of ABM in blood plasma; 2) "effective" (EA) and total (TA) albumin concentration in blood plasma; 3) quantitative parameters of albumin auto-fluorescence.

Screening of the individuals with diabetes mellitus 25-26 years after their work in Chernobyl revealed two groups of patients differing in structural/functional properties of albumin, first of all in conformations of plasma albumin and characteristics of the tryptophanyl region of molecule. The revealed structural modifications of membranes are dependent on radiation- induced factors. Concomitant diseases (diabetes mellitus, cardio-vascular diseases) reinforce radiation-induced effects in accordance with their manifestation. The obtained results clarify a heterogenous nature of albumin molecules and reveal quantitative differences of their conformation in the observed groups of patients. An albumin molecule in diabetics is modified in the chronic hypoxia conditions provoked mainly by the hyperglycemia and oxidative stress.

ABM is a sensitive probe of albumin alterations, and can be used to elucidate the changes in protein systems. Significant differences in albumin dynamics exist between the control group (donors), on the one hand, and diabetics and non-diabetics groups of Chernobyl clean-up workers, on the other. The biomarker of modified albumin could also help to identify individuals with complex metabolic conditions who have a higher risk of suffering from cardiovascular diseases.

Conclusions

Spectral parameters of ABM reflect a wide range of interrelated characteristics of lymphocyte membrane (physical-chemical state and microviscosity); proliferating and lipid metabolic activity of cells; phenotypical characteristics of lymphocytes; distribution of cells among subsets. The discovered significant alterations in ABM fluorescence characteristics in plasma could be explained, in part, by a diminished binding capacity and conformational changes of albumin in the observed groups of patients. The ABM fluorescence in cell suspension and blood plasma was found to correlate with select immunological parameters (CD4+/CD8+ ratios, t-lymphocyte counts, etc.).

There was a strong agreement between changes in ABM spectral characteristics and both clinical and pathological estimates. The observed changes of the studied ABM fluorescence parameters reflect alterations of the cellular mechanisms of immunity, which highlights application of the probe ABM for a preliminary screening test in immune diagnostics. Measurement of ABM spectral characteristics could potentially be a useful tool for estimating the immune state of patients. Compared to many commonly used diagnostic protocols, the fluorescence-based method is sensitive, less expensive and time-consuming, technically simple and convenient.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	IK	EK	TZ	GG	GK
Research concept and design	✓	✓	--	---	--
Collection and/or assembly of data	✓	✓	✓	--	--
Data analysis and interpretation	✓	✓	✓	✓	--
Writing the article	✓	✓	--	--	--
Critical revision of the article	✓	✓	✓	✓	✓
Final approval of article	✓	✓	✓	✓	✓
Statistical analysis	--	--	--	--	✓

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