ALTERATIONS OF ALBUMIN IN CHERNOBYL CLEAN-UP WORKERS BLOOD PLASMA AFTER MYOCARDIAL INFARCTION AND GROUP WITH EPILEPSY PAROXYSM

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INTRODUCTION

Around 6000 inhabitants (20 – 49 years old in 1986) of Latvia took part in clean-up work in Chernobyl from 1986 till 1991. Most of them were officially documented as recipients of ionizing radiation exposure (1–50 cGy). Exposure of biomolecules to ionizing radiation results in the damage that is initiated by free radicals and progresses through a variety of mechanisms. Radiation-induced cell membrane damage may be a consequence not only of lipid peroxidation but also of protein alterations.

The use of hydrophobic fluorescent probe ABM (benzanthrone derivative) and albumin autofluorescence allowed show conformational alterations in Chernobyl clean-up workers blood plasma. Patterns of ABM spectra had never been previously seen in examined healthy individuals or patients with tuberculosis, multiple sclerosis, rheumatoid arthritis, etc. Patterns of ABM fluorescence spectra are associated with conformational changes of blood plasma albumin. The use of probe ABM and albumin auto-fluorescence allowed show conformational alterations in albumin of Chernobyl clean-up workers blood plasma. It is necessary to note that all investigated parameters significantly differ in observed groups of patients. These findings reinforce our understanding that the blood plasma albumin is a significant biological target of radiation. It may be concluded that fluorescence characteristics are representative of radiation induced albumin alterations and its carrier function.

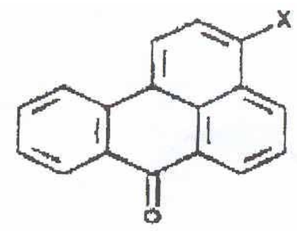

Fig. 1. The chemical structure of flurescent probe ABM

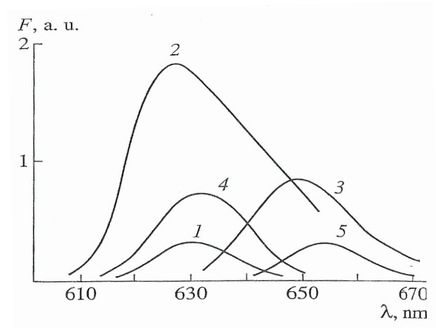

Fig. 2. ABM binding with human serum albumin (HAS)
Spectral characteristic of ABM in HAS (λex 485 nm)
1 - pH 1 - 2; 2 - pH 3.6; 3 - pH7.4; 4 - pH9.0; 5 - pH>11.5
Albumin concentration of probe in sample 10μM.

Changes of pH in the region 3 - 12 strongly affected the quantum yield and maximum position of fluorescence of ABM bound with albumin. Areas of greatest changes correspond to the known conformational transitions in proteins. ABM can be used as a probe which is sensitive to conformational changes of albumin.

AIM, MATERALS AND METHODS

It is widely accepted that the dynamics of plasma proteins (e.g. albumin) play a prime role immune characteristics of humans. The aim was to determine the several aspects of blood plasma albumin alterations in Chernobyl clean-up workers in relation with patients having no professional contact with radioactivity. For the ditailed study were selected the following groups of patients: (1) Group 1 – common group of clean-up workers without myocardial infarction (MI) or epilepsy (EP) in anamnesis (n=54); (2) Group 2 – clean-up workers with EP (n= 10); and (3) Group 3 with EP – having no professional contact with radioactivity; (4) Group 4 – clean-up workers after MI (n=10); (5) Group 5-with after MI (n=10); (6) Group 6 – healthy donors – having no professional contact with radioactivity . We detected spectral parameters of probe ABM (benzanthrone derivative) in blood plasma, albumin auto-fluorescence; albumin total (TA) and effective (EA) concentrations. Fluorescence parameters were registered on spectrofluorimeter Spectrofluo JY3 (ISA Jobin Yvon Instruments S.A., France) at excitation wavelength 470 nm and emission wavelength of 500 – 700 nm. Fluorescence intensity (F) was measured in arbitrary units (F, a.u.). In albumin auto-fluorescence investigations fluorescence spectra were measured at the excitation wavelength 286 nm. It is known the human plasma albumin fluorescence is dominated by tryptophane residues emission (330 nm).

Effective albumin concentration is “healthy” albumin equivalent in blood plasma, measured by fluorescent method (in this case using probe ABM). Reserve of albumin binding capacity was determined as „effective” albumin concentration (EA)/total albumin concentration (TA).

RESULTS AND DISCUSSION

COMMON GROUP OF CHERNOBYL CLEAN-UP WORKERS (Table 1)

In human blood plasma ABM is non-covalently bonded to albumin and has one emission maximum at 650 nm. The 1996–1997 data for the studied patients were similar to those obtained at pH 1–2: the fluorescence zone shifted to the short wave region (600 – 630 nm) as compared to the spectrum at pH 7.4 (650 nm); fluorescence intensity decreases by 24% in comparison with healthy donors. Screening of ABM labeled blood plasma samples in 2006–2008 revealed that in 83% of patients (Group 1) the fluorescence zone had shifted to the short wave region, but in 17% (Group 2) it had not changed (contrary to the spectrum for healthy donors).

We obtained 3 patterns of ABM fluorescence spectra in observed groups of patients:

1A. Fluorescence zone shifted to short wave region by 20 – 30 nm (620 – 630 nm).

1B. Fluorescence zone shifted to short wave region by 30 – 50 nm (600 – 620 nm).

2. Fluorescence maximum at 650 nm observed for blood plasma of healthy donors.

These observations may be consistent either with the changed binding of ABM or conformational alterations of albumin molecule. Level of structural heterogeneity could also follow a non-uniform pattern of fluorescence spectra significantly differing in groups of patients. Not so significant albumin structure alterations are obtained in Group 2 as compared with Group 1. Data in Group 1 results in splitting of albumin alterations onto two stages:

Group 1 (A). Acidic expansion stage (620–630 nm); fluorescence intensity decreases more significantly (by 17% as compared to the results obtained in 1996–1997).

Group 1 (B). N-F transition stage (600 – 620 nm). This well-known N-F transition takes place at pH values, lower than 4.3 and probably under ionizing radiation, and involves separation of domain III from the rest of the albumin molecule as well as the separation of the subdomains of domain III from each other (a change from a native or “N” form to a faster or “F” migrating form of albumin). The F form is characterized by a substantial increase in viscosity and much lower solubility. In our experiments, this stage is characterized by an increase of fluorescence intensity as compared to healthy donors (Table 1). It may be explained by an increased capacity of albumin binding sites. In human serum albumin for ligands exists multiple binding sites differed in affinity for this probe.

In acidic expansion stage relaxation of protein groups and bound water becomes faster. ABM binding sites are gradually being exposed to a more hydrophilic environment which leads to a reduction of fluorescence intensity. ABM fluorescence is significantly enhanced when it is adsorbed into hydrophobic sites in albumin after N-F transition. Affinity for the probe decreased, but due to a bigger number of binding sites the fluorescence intensity of ABM increased.

PATIENTS AFTER MYOCARDIC INFARCTION AND WITH EPILEPSY PAROXYSM (Table 2, 3)

Albumin is single of ABM fluorescence in human blood plasma (650nm). In Chernobyl clean-up workers (1B – common group without myocardial infarction and epilepsy; 3 – patients with epilepsy; 5 – after myocardial infarction) the patterns in spectral characteristics of ABM resemble so-called N-F transition. Blue shift of fluorescence maximum to 602–620 nm is accompanied by increasing fluorescence intensity as compared by healthy control 1.35; 1.07; 1.48 times in groups 1B, 3,5 correspondingly. In patients with epilepsy and after MI who had no professional contact with radioactivity the ABM emissions wavelength was not changed but fluorescence intensity as compared with control value decrease 1.23 and 1.48 times, correspondingly.

The levels of pathological/pharmacological metabolites (fatty acids, antioxidants, plasma levels of lipid peroxidation products etc.) in patient`s blood increased and their albumin could not ultimately bind them all. The above parameters balance differs in groups comparable to controls and hence their correlation to seizures pathophysiology and their degree. Metabolites caused conformational changes in albumin molecule and results as shifts away from binding sites with high affinity for the probe to other binding sites with lower affinities and specifities. Such shifts are in agreement with results of albumin auto-fluorescence data and ABM binding sites characteristics (compactability of albumin globule, binding constant, albumin effective concentration, albumin binding reserve etc.). A result clarifies the heterogeneous nature of ABM binding and reveled quantitative diferent conformation of albumin in observed groups of patients. The more pronounced albumin structural/functional alterations were observed in clean-up workers with EP and after MI. Results taking in account the negative dynamics of EEG indicates the multifunctional CNS pathology state in relation with disturbances in regulation mechanisms of neuroendocrine and immune systems. Therefore seems likely that external radiation and incorporated radionuclide’s predominate in alterations of albumin.

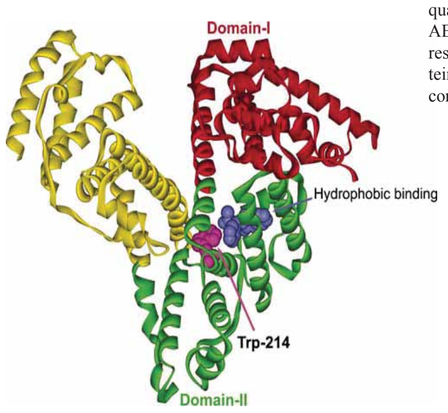

Fig. 3. The structure of human serum albumin and the location of tryptophan residue

Table 1. Spectral characterization of albumin in Chernobyl clean-up workers blood plasma (1996-2006)

Groups of clean-up workers	Probe ABM		Albumin Auto Fluorescence	
	Fluorescence emission max, nm	F, a.u	Fluorescence emission max., nm	F, a.u.
1 ^{A} (N-F transition)	600-620	2.85± 0.07	309-311	1.85±0.06
1 ^{B} (acidic expansion)	620-630	1.24± 0.07	311-318	2.15±0.06
2 group	650	1.74±0.06	330	2.48±0.04
3 Control group (healthy donors)	650	2.11±0.06	330	2.96±0.05
p between groups		1-1; 1^{A}-2 1^{A}-3, 1^{B}-2 1^{B}-3, 2-3		1-1; 1^{A}-2 1^{A}-3, 1^{B}-2 1^{B}-3, 2-3

Notes: Blood plasma diluted 200 fold; ABM concentration in the sample. - 19.6 nmol/ml; Albumin auto-fluorescence excitation 286 nm;
F - fluorescence intensity (arbitrary units)

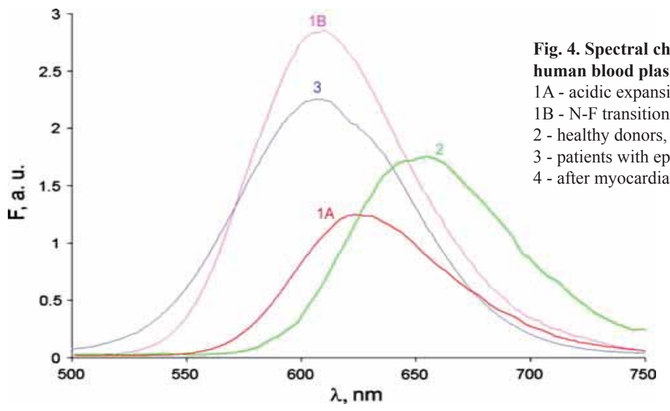

Fig. 4. Spectral characteristics of ABM in human blood plasma:
1A - acidic expansion;
1B - N-F transition;
2 - healthy donors,
3 - patients with epilepsy;
4 - after myocardial infarction (MI)

Table 2. Spectral ccharacterizations of blood albumin patients’ groups (2006-2008)

Groups of patients	Probe ABM		Albumin Auto- Fluorescence	
	Fluorescence emission max, nm	F, a.u	Fluorescence emission max., nm	F, a.u.
1. Common group of clean-up workers (without epilepsy and myocardial infarction in anamnesis) n=54	600-620	2.85± 0.07	309-311	1.85±0.06
2. Epilepsy (Chernobyl clean-up workers n=10)	604-614	2.25±0.07	309-311	1.38±0.07
3. Epilepsy patients (n=19)	650	1.71±0.05	-	-
4. MI (Chernobyl clean-up workers, n=10)	602-611	3.13±0.05	309-311	1.01±0.05
5. MI patients (n=17)	650	1.43±0.06	-	-
6. Control (n=17)	650	2.11±0.06	330	2.96±0.05
p<0.05 (between groups)		1-2, 1-3, 1-4, 1-5, 1-6, 2-3, 2-4, 2-5, 2-6, 3-4, 3-5, 3-6, 4-5, 4-6		1-2, 1-4, 1-6, 2-4, 2-6 2-4, 2-6, 4-6

Table 3. Characteristic of blood plasma albumin binding sites in patients groups (2006-2008)

Groups of patients	Effective concentration of albumin in blood plasma (EA)(g/l)	Total concentration of albumin in blood plasma (TA) (g/l)	Reserve of albumin building capacity (EA/TA)
1 Common group of clean-up workers (without epilepsy and myocardial infarction in anamnesis) n=54	49.8±2.6	75.1±1.3	0.66±0.03
2. Epilepsy (Chernobyl clean-up workers n=10)	41±1.6	69±1.5	0.60±0.05
3. Epilepsy patients (n=19)	49.7±1.3	74±1.4	0.67±0.04
4. MI (Chernobyl clean-up workers n=10)	38.1±1.8	71.6±1.4	0.53±0.02
5. MI patients (n=17)	46.9±1.2	75.1 ±1.1	0.62±0.03
6. Control (n=17)	68.0±3.4	83.4±1.2	0.81±0.04
p<0.05 (between groups)	1-2, 1-4, 1-6	1-2, 1-6	1-2, 1-4, 1-5, 1-6

Note: Plasma diluted 200X, ABM concentration in sample 19.6 nmol/ml, **F**-fluorescence intensity in arbitrary units. **TA**-total concentration of albumin in plasma; EA- equivalent of “healthy” albumin in plasma; **EA/TA**- reserve of albumin binding capacity; Values shown are in mean (±SE)

CONCLUSIONS

Obtained patterns of ABM fluorescence spectra suggest that various qualitative changes are evident in blood plasma albumin of Chernobyl clean-up workers in comparison with previously examined healthy donors or patients having no professional contact with radioactivity.

Both of phospholipids and the total fatty acid values showed significantly greater variability in the epileptics and patients after myocardial infarction than in non-epileptic subjects and patients without myocardial infarction in anamnesis.

Changes in albumin structural/functional properties in observed patients groups’ primary were due to biochemical changes in plasma lipids.

Reults clarify the heterogeneous nature of ABM binding and revealed quantitative different conformation of albumin in observed groups of patients. The more pronounced albumin structural/functional alteration was observed in clean-up workers with epilepsy and after myocardial infarction. Results taking in account the negative dynamics of EEG indicate the multifunctional CNS pathology state in relation with disturbances in regulation mechanisms of neuroendocrine and immune systems. Therefore seems likely that external radiation and incorporated radionuclide predominate in alterations of albumin.

Fluorescence characteristics of ABM in blood plasma and albumin auto-fluorescence data are representative of radiation induced albumin structural/functional alterations. Fluorescence – based methods are very sensitive, technically simple and not time-consuming in comparison with absorption- based methods.

CORRELATIONS

There is a strong correlation between the investigated parameters such as spectral characteristics of ABM in blood plasma, albumin auto-fluorescence, on the one hand, and tryptophanyl region dehydration of albumin molecule, on the other.

ABM spectral characteristics in plasma and cells correlate with clinical and pathological investigations.

ABM fluorescence intensity correlates with "effective" albumin concentration, value of albumin binding reserve and probe binding sites characteristics (its polarity, dehydration of tryptophanyl region of globule etc.)

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