

Butyrylcholinesterase in UV Reactions

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The authors analyzed butyrylcholinesterase status in melanocytic pathology and possible association of enzyme activity with melanoma diagnose and progression. Butyrylcholinesterase activity showed no changes between melanoma and dysplastic nevi, between melanoma and control, between nevi and control, but it had a statistically significant variation between primary and metastatic melanoma. Butyrylcholinesterase activity had statistically significant variations with site of tumor ($p=0.001$), with histological type ($p=0.0017$), with Clark level ($p=0.007$) and with the presence/absence of ulceration ($p=0.004$). We analyzed the variations of butyrylcholinesterase activity by markers for melanoma staging and progression in primary and metastatic melanoma. Significant correlations were recorded between butyrylcholinesterase and melanoma progression. The authors obtained a negative correlation between butyrylcholinesterase activity and CRP, between butyrylcholinesterase and malondialdehyde. A positive correlation was observed between butyrylcholinesterase and albumin in patients with metastatic melanoma.

Keywords: butyrylcholinesterase, LDH, CRP, MDA, cutaneous melanoma

The body possesses enzymes able to split esters of choline with various organic acids. Some authors have described two main classes of cholinesterase: acetylcholine acylhydrolases (EC 3.1.1.7), also called acetylcholinesterase, and choline acyl-acylhydrolases (EC 3.1.1.8), also called nonspecific esterases or pseudocholinesterases. These enzymes (1-14) are distinguished by genetic polymorphism, substrate specificity, susceptibility to competitive inhibitors, enzymatic kinetic, biological role, tissue distribution and sensitivity to some enzyme modulators. Acetylcholinesterase can be found in cholinergic neurons, both in cell body and in dendrites/axonal endings, and also in other tissues (lung, spleen, erythrocytes). Acetylcholinesterase has an essential role in the inactivation of neurotransmitters and in the modulation of neuromuscular transmission of impulses. Acyl-choline-acylhydrolases, found in liver, pancreas, heart and nervous system (white substance) are actively secreted in plasma and act on acetylcholine, butyrylcholine, benzoylcholine, succinylcholine. The level of pseudocholinesterases gives us information about the proteosynthesis capacity of liver. Atypical cholinesterase (1,2,3,14) differs from the normal enzyme through resistance to some inhibitors, such as dibucaine or fluorine. Atypical form of cholinesterase has a reduced capacity to hydrolyze succinylcholine, a preparation used in anesthesia as a muscle relaxant.

Cholinesterase biological functions has been studied since the beginning of the 20th century, as multiple clinical situations have demonstrated the utility of cholinesterase. Determination of serum cholinesterase is valuable in monitoring patients with atypical forms of this enzyme, poison with organophosphates, acute infections, pulmonary embolism, muscular dystrophy, metabolic syndrome, myocardial infarction and neoplastic states (4-16). Butyrylcholinesterase (BChE) is the most discussed and described enzyme of this class (6,13,14,17,18), which catalyzes the hydrolysis of butyrylcholine in thiocoline and butyric acid. This enzyme is a serine-esterase, identified in the liver, skin, lungs, heart, brain, small intestine, kidney,

adipose tissue and plasma. BChE and AChE use a catalytic triad mechanism for catalysis, similar to that used by serine proteases (6,18). BChE is involved in maintaining the balance between proliferation and cell death, as well as in protecting cells from drug-induced cytotoxicity.

The authors aimed to evaluate: i) BChE status in patients with melanocytic lesions (benign and malignant tumours), ii) the association between BChE and progression of malignant melanoma, iii) the effect of inflammation on BChE activity.

Experimental part

Materials and methods

We made a study that included melanocytic pathology (dysplastic nevi, malignant melanoma) diagnosed, histopathologically and / or immunohistochemically.

Inclusion criteria: untreated melanocytic lesions

Exclusion criteria: obesity, diabetes, endogenous hypertriglyceridemia, hypothyroidism, hyperthyroidism, acute infections, pulmonary embolism, muscular dystrophy, myocardial infarction, cachexia, poisoning with organophosphates, nephrotic syndrome, severe anemia, other malignancies, pregnancy, patients with long-term medication, people with atypical cholinesterase phenotypes.

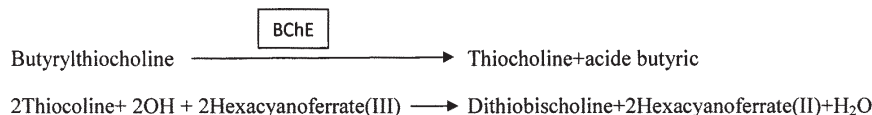
The study was carried out from 2008 to 2012 (106 primary melanoma stages I si II and 22 metastatic melanoma stages III and IV according to AJCC) with primary melanoma and 48 with dysplastic nevi. Control group consisted of 48 healthy volunteers.

Laboratory data. Hematological determinations were made with ABX Pentra 60 automated analyzer (ABX Diagnostics France). Biochemical determinations (spectrophotometry/turbidimetry) were performed with HumaStar Analyzer (Human GmbH, Wiesbaden, Germany). Serological parameters were determined by ELISA method (HS Reader Huma Germany). Butyrylcholinesterase and lactatedehydrogenase (LDH) activity were determined by spectrophotometric methods. Quantitative determination of C reactive protein (CRP) was made by turbidimetric

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method. Malondialdehyde was quantitatively determined using thiobarbituric acid (19).

Principle of method. BChE catalysis butyrylcholine to thiocholine and butyric acid. Enzymatic activity is determined by the decrease rate of hexacyanoferrate(III) measured at 405nm, by the reactions catalysed by cholineoxidase and peroxidase.



Sample materials were the venous blood collected in anticoagulant for hematological measurements and serum for biochemical and serological determinations.

Statistical analysis. All statistical procedures were performed using SPSS. Differences between patients and controls were compared using Pearson's chi-square test. Comparisons between groups were made using linear regression. Results are presented as percentage, mean, median and standard deviation. Statistical significance was set at 5% (20).

Results and discussions

Butyrylcholinesterase status in people with melanocytic proliferations. Enzymatic activity of BChE in adult patients with melanoma, in people with dysplastic nevi and healthy volunteer was presented in figure 1. Low BChE values were obtained in 22 (17.18%) patients with metastatic melanoma, after the evaluation made by investigators. No statistically significant variations of BChE were obtained in patients with melanoma compared with control, respectively, melanoma group compared with dysplastic nevi group (table 1). Statistically significant differences were observed in BChE activity when compared primary melanoma and metastatic melanoma ($p < 0.01$).

Butyrylcholinesterase activity and progression of melanoma. The BChE activity was analyzed according to predefined stratification variables used as predictive factors of melanoma. There were taken into account: sex, clinical

and histopathologic features (Breslow index, Clark level, histological type, primary tumor, absence/presence of ulceration), and markers for disease staging. In table 2 we presented the mean and standard deviations of BChE activity in patients with melanoma, grouped by clinical and histopathological criteria. BChE activity did not vary with gender. BChE activity had statistically significant variations with site of tumor ($p = 0.001$), with histological type ($p = 0.0017$), with Clark level ($p = 0.007$) and with the presence/absence of ulceration ($p = 0.004$). BChE levels did not vary with Breslow index.

In table 3 we synthesized mean values and standard deviations for BChE, LDH, CRP, MDA and albumin in patients with primary and metastatic melanoma. Statistical analysis revealed significant correlations between BChE activity and biochemical factors for progression and staging of melanoma.

Inflammation and oxidative stress influence on the activity of butyrylcholinesterase. Synthesis of acute phase reactants is stimulated in patients with melanoma. We obtained a negative association of statistical significance between BChE activity and C-reactive protein (table 3). The strong positive association obtained between BChE and albumin was statistically significant. The negative correlation between malondialdehyde and BChE is also significant.

Toxicological and pharmacological importance of BChE had been extensively studied, but the physiological role of the enzyme was not exactly established. Some studies showed that BChE, through its esterase activity, was scavenging of organophosphates and carbamate inhibitors, was regulating cholinergic transmission, inactivated some drugs (cocaine, aspirin, amitriptyline), activated prodrugs to active forms (bambuterol, heroin) (6, 9, 15, 17, 24). BChE was involved in neurotransmitter homeostasis through acylamidase activity (6, 10, 17). Peptidase activity of BChE played a central role in the production of β -amyloid and helped her diffuse in β -amyloid plaques (6, 9, 17, 23). Expression and abnormal BChE activity were observed in different human cancers, suggesting its possible involvement in tumorigenesis and metastasis (6, 7, 9). Experimental and clinical studies reported that BChE was involved in neurotransmitter regulation, interaction between non-neuromuscular cells, proliferation and cell differentiation, cell regeneration efficiency, tumorigenesis,

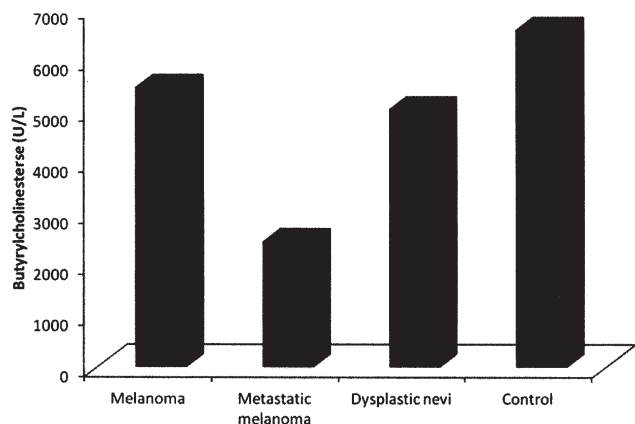


Fig. 1. Butyrylcholinesterase activity in participants in the study

Groups	LDH(U/L)	BChE (U/L)	Albumin (g/dl)	MDA (umol/L)	CRP (mg/dl)
Primary melanoma	306±118	5475.85±1774.15	4.03±0.41	288±117	0.37±0.28
Metastatic Melanoma	566±104	2462.77±1460.39	3.17±0.66	326±104	0.73±0.61
Dysplastic nevi	288±81	5038.31±1736.77	4.29±0.27	199±61	0.11±0.11
Control	274±66	6572.73±1365.96	4.22±0.38	203±74	0.16±0.16

Table 1
BIOLOGICAL PARAMETERS IN PATIENTS WITH MELANOCITYC LESIONS AND CONTROL

Parameter	Butyrylcholinesterase (U/L)	p
Sex		
Male (n=54)	5500.96±1851.57	p=0.78
Female (n=74)	5458.68±1731.47	
Site of tumor		
Head-neck (n=11)	5065.71±1360.26	p=0.001
Trunck (n=83)	5000.79±1761.37	
Limbs (n=86)	6417.65±1449.97	
Unclassified	6809.69±1255.56	
Hystological type		
Nodular melanoma (n=61)	4477.30±1235.20	p=0.0017
Extensive in surface melanom (n=21)	6628.22±1308.18	
Lentigo malignant melanoma (n=22)	6146.45±2377.86	
Acral lentigous melanoma (n=9)	6521.77±1260.46	
Unclassified melanoma (n=15)	6146.86±1372.18	
Breslow index		
0-1.0 mm (n=22)	6628.22±1308.18	p=0.13
1.01-2.0 mm (n=34)	6889.97±1577.20	
2.01 – 3.0mm (n=16)	5460.62±1133.15	
3.01-4.0mm (n=36)	4232.38±1010.76	
>4.01mm (n=20)	4054.70±1326.58	
Clark level		
II (n=27)	6482.77±1257.84	p=0.007
III (n=47)	6082.14±1916.09	
IV (n=36)	4742.16±1275.24	
V (n=18)	3849.77±1116.30	
Ulceration		
Ulcerated melanoma (n=17)	3538±1071.74	p=0.040
Unulcerated melanoma (n=108)	5752.59±1681.64	

Results (mean±SD)were grouped related to melanoma characteristics.p<0.05 has statistical significance for IC=95%.

Table 2
BUTYRYLCHOLINESTERASE
ACTIVITY STRATIFIED BY
CLINICAL AND
HISTOPATHOLOGICAL
CHARACTERISTICS IN PATIENTS
WITH MELANOMA

Variable	Primary melanoma	Metastatic melanoma
LDH	r=0.06	r=0.63
(U/L)	p=0.04	p=0.02
CRP	r= - 0.16	r= - 0.06
(mg/dl)	p=0.04	p=0.03
Albumin	r= 0.25	r= 0.417
(g/dl)	p=0.15	p=0.01
Malondialdehyde	r= - 0.48	r= - 0.78
(umol/L)	p=0.04	p=0.02

Results were considered statistically significant when p<0.05,IC=95%

Table 3
CORRELATION BETWEEN
BUTYRYLCHOLINESTERASE
ACTIVITY, AND STUDIED
PARAMETERS IN MELANOMA
SAMPLES

cell migration and adhesion, immune response, systemic inflammation, apoptosis, detoxification (1,6, 7, 9-12). BChE proved to be a useful biomarker in head and neck cancer, uterin cervix cancer, malignant disorders of the oral mucosa, meningioma, glioma, acoustic neurinomas, lung cancer, megakaryocytic disorders, leukemias, ovarian tumours, neuroblastoma, colon cancer, osteosarcoma, multiple myeloma, chronic liver disease, malignancies treated by radiotherapy and chimiotherapy (2,4,5,7-9, 11, 12, 17, 23).

Using BChE in diagnose and monitoring patients with melanoma could be based on the following results:

1. Altered activity of BChE is a nonspecific change in primary melanoma, but is significantly decreased in metastatic melanoma (fig. 1);

2. We did not found any statistical differences between BChE activity in patients with melanoma versus displastyc nevi or control (table 1);

3. Statistically relevant correlations were determined between BChE activity and factors for melanoma progression recognized by the American Joint Committee on Cancer (table 3).

4. In metastatic melanoma we obtained a positive correlation between BChE activity and albumin (table 3).

A series of clinical and experimental data suggested alterations of BChE activity in pathological situations characterized by low grade systemic inflammation, by enhancing angiogenesis and cell proliferation, by altering antioxidant defense system (1,10-13, 17, 18, 21-26). It outlined the special complexity of the factors that influence the activity of BChE in patients with melanoma. The authors found that the acute phase reactants and oxidative stress induced changes in activity of BChE in patients with melanoma. This conclusion was based on the existence of negative correlations with statistical significance between BChE activity and acute phase reactants (Table 4). The most plausible explanation for BChE-albumin correlation was that serum BChE and albumin levels were determined by their rate of hepatic production (17,25). BChE and albumin could be considered negative acute phase reactants, whose serum levels tended to decrease in inflammation, contrary to positive acute phase reactants (25). Results obtained in this study were consistent with those reported recently according to which (26) parameters of oxidative stress (thiobarbituric acid reactive substances, catalase, superoxide dismutase and glutathione peroxidase) and inflammatory markers (interleukin 6, C reactive protein, tumor necrosis factor- α and nitrites) influenced the activity of BChE.

Conclusions

Based on these findings, the authors conclude that BChE status is not a useful marker in the management of patients with primary cutaneous melanoma, but BChE can be used as factor of tumoral metastasis. BChE activity is influenced by factor of staging and progression of melanoma.

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