Chapter

Role of Growth Factors and Apoptosis Proteins in Cognitive Disorder Development in Patients with Duchenne Muscular Dystrophy

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Abstract

Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disease; it occurs due to a mutation in the dystrophin protein gene; as a result, the protein is not synthesized and muscle tissue dies. On the one hand, we can say that this disease has been sufficiently studied; however, it is still incurable, and there are a number of issues remaining unclear in terms of the development of progressive dementia as a symptom in 30% of patients with Duchenne muscular dystrophy. We conducted a study at the intersection of molecular genetic, neurological, and enzyme-linked immunosorbent patients' blood tests and experiments in organotypic culture, which allowed us to determine important points in the development of cognitive disorders in patients with Duchenne muscular dystrophy and identify a significant effect of growth factor concentration in patients. The chapter will present data on neurotrophic regulation in patients with Duchenne muscular dystrophy (by the best-studied neurotrophins), demonstrate special aspects of neuron-myocyte interaction, and broaden the understanding of the role of apoptosis and synthase proteins in the development of this disease. We would like to highlight the importance of prognostic criteria for the development of cognitive impairment and possible therapeutic measures to prevent progressive dementia

Keywords: Duchenne muscular dystrophy, cognitive disorders, pathogenesis, mutations in dystrophin gene, brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), immunoenzyme method, blood serum

1. Introduction

Nowadays, researchers have greatly succeeded in understanding the pathogenesis of Duchenne muscular dystrophy (DMD); numerous studies resulted in drugs that can treat some forms of DMD, affecting the course of the disease, converting it from a malignant form to a benign one, and thus increasing locomotion ability of patients

[1]. However, the study of cognitive impairment pathogenesis in DMD patients and a search for drug therapy for these disorders remain an important issue. It is a well-known fact that genetically DMD is caused by mutations in the gene encoding the dystrophin protein, which is a part of the dystrophin-glycoprotein complex and appears both in muscle and nerve cells, being involved in functioning of voltage-dependent channels and in synaptogenesis [2]. The gene encoding the dystrophin protein is the largest human gene, has 5 promoters, has 80 exons, is 24,000 kilobase long, and encodes a 427 kilodaltons protein. [3]. One of the key characteristics of dystrophin is its large number of tissue-specific isoform pairs. So far, researchers have identified more than a dozen isoforms expressed by internal promoters, Dp427, Dp260, Dp140, Dp116, and Dp71, and located in various organs: the lymphocytes, kidneys, cerebral cortex, cerebellum, peripheral nerves, Schwann's sheath, and retina [4–7]. The dystrophin gene has a high frequency of mutations: both point mutations as nucleotide substitutions and extended mutations as deletions and recombinations [8, 9]. Deletions are unevenly distributed along the gene length; they are more likely to be found in hotspots: 50–52 or 42–44 exons. Mutant protein forms in the body lead to dysfunctions of the dystrophin-glycoprotein complex and muscle-nervous system [10]. It is believed that a changed expression of the Dp140 isoform is one of the factors leading to cognitive impairment development in patients with DMD [11]. For example, researchers found that DMD patients with a mutation in promoters of the Dp140 and Dp71 isoforms have IQ index lower than in the case of Dp260 and Dp116 promoter mutations [12]. Still, some researchers associate frequency and severity of mental retardation in DMD patients with the absence of several dystrophin isoforms encoded in the distal end of the gene [13]. Although dystrophin gene mutations have long been recognized as a cause of mental retardation in DMD patients, there are cases when such patients do not have a pronounced cognitive deficit [14].

So, there is still much to be discovered about the occurrence mechanism of cognitive disorders in DMD patients, and some issues are still open to debates. We think that cognitive impairment in DMD patients relies on a combination of mechanisms, leading to a cognitive defect. Among such mechanisms are well-studied molecular genetic factors, distal location of the mutation in the DMD gene or effect from various combinations of impaired synthesis of DMD protein isoforms (Dp140, Dp116, Dp260, and Dp71); on the other hand, none the less important are biochemical factor-associated neurotrophic regulation and apoptosis mechanisms, but these factors are not so well-studied. Regrettably, things we know about physiology of higher nervous activity in terms of neurotrophic regulation are not enough for a comprehensive picture to explain the role of growth neurotrophic factors in cognitive disorder development. However, we know that neurotrophic factors, being polypeptide compounds, are synthesized by neurons and glia cells, get involved in the regulation of growth and differentiation processes, and ensure viability of the nervous tissue and its functions, both in terms of individual neurons and the whole nervous system [15]. Neurotrophins are also involved in synaptic plasticity regulation; they are known to form neuron cytoskeleton, new synapses, and receptors, and they are important for structural ordering of neurons or neuronal groups [16]. Researchers found that polypeptide growth factors are involved in the growth of axons and dendrites, trophic membrane receptors, release of neurotransmitters, and functioning of synapses. So far, the most well-studied neurotrophins, with a very similar structure, are nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Neurotrophic factors also include two subfamilies: glial cell-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF). In the human body, neurotrophins are synthesized by a target cell, then diffuse toward the neuron, and bind to receptor molecules on its surface, which causes an active axon growth (sprouting) and dendritic branching (arborization) [17, 18].

So, the axon reaches a target cell and comes into a synaptic contact. Neurotrophins can act both locally, within the same cell population, and remotely, circulating with the blood flow [19]. Neurotrophins make their effect on a neuron through a contact of polypeptide ligands with tyrosine kinase receptors (Trk-A, Trk-B, Trk-C) and low-affinity p75 receptor [20–22]. Neurotrophins trigger a regulating mechanism of cell growth and differentiation by activating a protein kinase cascade called mitogen-activated protein kinase, the MAP kinase pathway [23]. The pathway is activated by Grb2 tyrosine phosphorylation; the Grb2 is a protein that contains SH2 and S3 domains (sre homology region). After a series of cascading reactions, the phosphorylated MAP kinase goes through a nuclear membrane and phosphorylates various gene transcription factors in the nucleus. The resulting gene transcription changes trigger proliferation, differentiation, and maintenance of neuron viability.

NGF is a trophic factor essential for survival and differentiation of nerve cells in the central and peripheral nervous system; it binds to the low-affinity p140 receptor and the high-affinity tyrosine kinase receptor (Trk-A). Myocytes and neurons serve as target cells and secrete neurotrophin, which then binds to receptor ligands on the cell surface, gets captured by a neuron, undergoes endocytosis, and gets retrogradely transported to the nerve cell soma. There, NGF directly affects the nucleus, by changing the generation of enzymes in charge of neurotransmitter synthesis and axon growth.

BDNF is a dimer with a total molecular weight of 27.2 kDa; its structure is similar to that of NGF. Like other neurotrophins, BDNF is involved in the development and survival of brain neurons, including sensory neurons, dopaminergic neurons of the substantia nigra, and cholinergic neurons of the forebrain, hippocampus, and retinal ganglia. Researchers found both mature BDNF forms and pro-BDNF precursors in the central nervous system [24]. It is interesting to study the relations of BDNF expression to the activity of glutamate receptors, commonly found in the central nervous system. It is supposed that BDNF controls the balance between glutamate and (GABA)-ergic systems and has multiple other functions; for instance, when the nervous system is developed, BDNF is involved in synapse formation as well as differentiation, maturation, and survival of neurons.

CNTF is a single-chain polypeptide with 200 amino acid residues and has a molecular weight of 22.7 kDa. It is involved in the survival and differentiation of nervous system cells. High concentrations of CNTF can cause apoptosis. Some researchers believe that CNTF is also involved in glial cell differentiation.

There are many researches that prove that growth polypeptides can be treated as key regulators of cognitive functions and memory retaining processes. Recently, it has been positively proven that NTF growth factors introduced into the brain parenchyma ensure the preservation of brain tissue in critical periods, by protecting neurons from the damaging effects of destructive agents [25–28].

2. Materials and methods

Our research clarified the role of growth factors and apoptosis proteins in cognitive disorder pathogenesis in patients with Duchenne muscular dystrophy. There were 36 male DMD patients aged 5–22 years (average age was 13.7 years) followed up by us in the clinic of North-Western State Medical University named after I.I. Mechnikov, in a stationary unit, and as part of the on-call service of the Saint Petersburg Children's Hospice. The control group consisted of 30 healthy people (7–22 years old, average age 13.8 years). Clinical, molecular genetic, and laboratory tests were carried out. The clinical and neurological examination was conducted according to a generally accepted protocol with inclusion of neuropsychological

testing to determine the severity of cognitive disorders. The following methods were used to test the memory: the method of memorizing 10 words; memorizing 9 geometric shapes; delayed reproduction of 10 words and 9 geometric shapes; and Muchnik-Smirnov "double test." Two methods were used for the thinking test, "comparison of concepts" and "directed verbal associations"; attention tests were performed using Schulte tables. We used adapted methods taking into account the age characteristics of DMD patients using a scoring system: memory, attention, and thinking were evaluated from 1 to 30 points, and then the test results were summed up. It helped to differentiate the identified cognitive disorders based on their severity into moderate and severe cognitive disorders (from 1 to 30 points, severe cognitive disorders; from 31 to 60, moderate disorders; and from 61 points to 90 points, no disorders). The search for deletions in the dystrophin gene was performed using multiplex PCR (20 exons and a promoter region). The search for deletions and duplications was performed using multiplex ligation-dependent probe amplification (79 exons and a promoter region). We used sets of probes P034 and P035 by MRC Holland (the Netherlands). The analysis was performed using an automatic capillary electrophoresis system ABI 3130×1 (Applied Biosystems, USA). The detection of point mutations was carried in Cochin Hospital (Paris, France) by next-generation sequencing with subsequent Sanger verification. Determination of the level of neurotrophins and apoptosis proteins is as follows: brain-derived neurotrophic factor, nerve growth factor and ciliary neurotrophic factor (CNTF), caspase 8 (K8), cytochrome C (CC), apoptosis-regulating proteins Bcl2 and p53 were performed using an enzyme immunoassay in blood serum samples. Enzyme immunoassay kits by RayBiotech, Inc. were used. The threshold values for the determination of BGF, NGF, and CNTF were 20 pg/ml, 14 pg/ml, and 8 pg/ml, respectively; K8, 0.10 ng/ml; CC, 0.05 ng/ml; and for Bcl2 and p53 proteins, 0.5 ng/ml and 0.33 U/ml, respectively. DMD patients were divided into two groups based on the presence of cognitive disorders: group 1, patients without cognitive disorders (n = 17); group 2, patients with moderate and severe cognitive disorders (n = 19). In order to clarify the role of the mutation location in the DMD gene, patients with confirmed mutation were divided into two groups based on the location of the mutation in the proximal section of the DMD X chromosome gene (from exon 1 to 40) (n = 8) or the distal section from exon 41 to 79 (n = 16). This distribution was caused by the data on the role of dystrophin protein isoforms expressed from the distal part of the Dp140 and Dp71 genes in the development of cognitive disorders in DMD patients.

The experimental study was carried out in the laboratory of excitable membranes of the FSBES Pavlov Institute of Physiology of the Russian Academy of Sciences. Methods of neural tissue culture, morphometric, immunochemical, and histological studies were used. In order to study the neurotrophic properties of blood plasma in the patients, 5 ml of venous blood was taken on an empty stomach in the morning. The blood was centrifuged, the separated plasma was transferred to a micro-tube and frozen at a temperature of −80°C. The effect of patients' blood plasma on the growth of spinal ganglia neurites was evaluated using the organotypic tissue culture method. An experimental model was based on 10–12-day-old chicken embryos, from which spinal ganglia were isolated at the level of the lumbosacral spine (L5-S1).

Spinal ganglia (explants) were placed on the bottom of a Petri dish covered with a collagen film. Each Petri dish contained 20–25 explants. In order to attach the explants to the collagen substrate, closed Petri dishes were placed in a thermostat at 36.8°C for 10 minutes, and then a nutrient medium was added. We used nutrient media with a pH of 7.4 of the following composition: 40% Hanks solution; 40% Eagle's medium; 15% veal embryonic serum, for cell cultures, HyClone; 5% chicken embryonic extract; with addition of glucose (0.6%), insulin (0.5 u/ml), gentamicin

(100 u/ml), glutamine (0.35%). Chicken embryonic extract was made of 10–12-day-old chicken embryos. Further cultivation of the spinal ganglia explants was performed at 37°C and 5% CO2 for 3 days in a CO2 incubator (Sanyo, Japan).

In order to study the patient's blood plasma parameters and further pharmacological analysis, 25–30 explants per studied concentration were used, taking into account that the experimental test consisted of 10 stages; the average number of explants per patient was 250–300 pieces. Same number of explants was used in the study of blood plasma of healthy people from the control group. Total number of explants studied during the experimental study was 19,000.

When testing blood plasma in tissue culture, blood plasma was added to experimental dishes at the dilution range from 1:100 to 1:2 (1:2, 1:10, 1:50, 1:70, 1:100). Control explants were cultured in a standard nutrient medium without an addition of blood plasma and in a medium with addition of blood plasma of healthy people in the same dilutions. Growth of neurites in tissue culture was studied in vivo using a light microscope and various stains and fluorescent agents. In order to evaluate the growth activity of neurites in the growth zone of the explant and analyze the data obtained, we used a relative criterion—the area index (AI), which was calculated as a ratio of the area of the entire explant, including the peripheral growth zone, to the initial area of the ganglion (AI = S (CA+GA)/S (CA)) (**Figure 1**).

The square of the ocular grid of the microscope was taken as a conventional unit of area (the side of the square at the magnification of 3.5 × 10 was equal to 150 microns). Control AI value was taken as 100%.

In order to clarify the biochemical mechanisms involved in pathological cascades in orphan inherited neuromuscular diseases, a test system was developed that included a sequential study of the patient's blood plasma in an organotypic tissue culture in a 1:70 dilution, followed by an addition of reagents to the medium: synthetic nerve growth factor (100 pg/ml) with subsequent cultivation for 3 days and AI calculation. An indirect immunohistochemical method was used to visualize the cytoskeleton of neurons in the spinal ganglia and their processes (neurites).

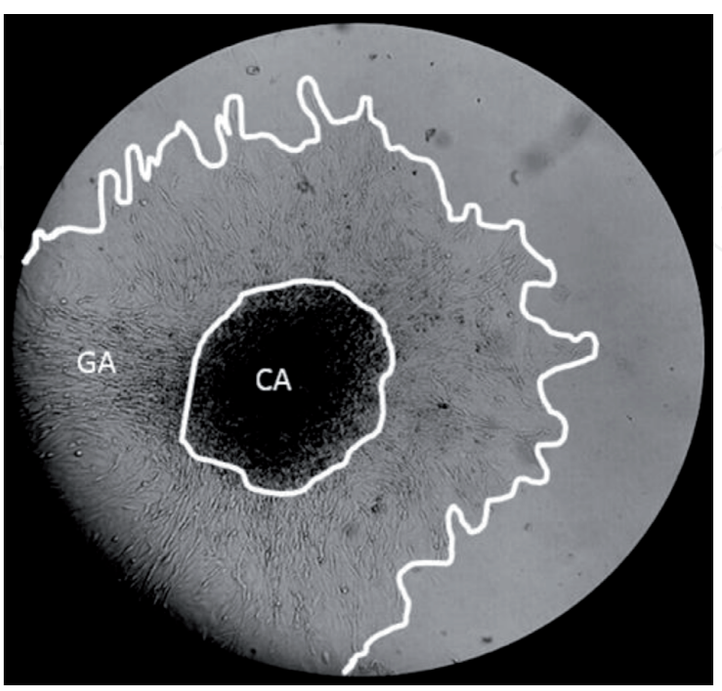

Figure 1.
Diagram of tissue explants of 10–12-day-old chicken embryo (3 days of cultivation) in an organotypic culture. (CA, central area; GA, growth area).

In order to obtain the lifetime information about the condition of cells forming in the growth zone of the spinal ganglia explants and the heart tissues, we used a hardware-software complex for visualization, processing, and analysis of images ZEN_2009 and ZEN_2014 based on laser scanning microscope LSM-710 (Carl Zeiss, Germany). Microscopic tests were performed using an equipment of the Center for Collective Use "confocal microscopy" of the I. P. Pavlov Institute of Physiology of the Russian Academy of Sciences. Visualization of the objects was made using Axiostar Plus microscope (Carl Zeiss, Germany). The resulting images were analyzed using the ImageJ software.

Statistical analysis was performed using STATISTICA 8.0 package (StatSoft®, Inc., USA, 2012). The following methods of statistical analysis were used: assessment of the effect of the quality factor on the variance of the quantitative metric using the analysis of variance, evaluation of the strength and direction of linear association between quantitative variables using parametric Pearson correlation coefficient, nonlinear relationships using Spearman correlation coefficient, conformity assessment according to the Shapiro-Wilk test, and determination of the numerical characteristics of variables. If the null hypothesis was rejected, the median, upper, and lower quartiles of Me [Q25; Q75] were used to test whether the empirical distribution law of a random variable corresponds to the theoretical law of the normal distribution. Quantitative characteristics were described using the arithmetic mean and the standard deviation. The null statistical hypothesis was rejected at a significance level of $p < 0.05$.

3. Results

Thirty-six male patients aged 5–22 years (average age 13.7 years) had Duchenne muscular dystrophy with motor disorders as myopathic syndrome, including generalized muscle weakness, hypotension, as well as pseudohypertrophy. Among the examined DMD patients, 63% had the disease debut at the age of 2–5 years, while 37% had a later onset, at 5–7 years. In 18% of cases, the disease was progressing rapidly, and 13% of patients required mechanical ventilation at the time of study. In all patients, the disease began with damage to muscles of the pelvic girdle and proximal legs and was steadily progressive. All patients had elbow, knee, and ankle joint contractures of varying severity; 56% of patients had spinal deformities of varying severity: scoliosis (25%), kyphoscoliosis (53%), and Friedreich's ataxia deformity. 68% of patients suffered from cardiopathy and 57% of patients from pneumopathy. EDSS scale grades the patients as follows: 22%, high disability degree (9.5 points), bedridden, with a tracheostomy and mechanical ventilation, and requiring full nursing care. 64% ranged from 8.0 to 9.0 EDSS points: they moved in a wheelchair (motorized), retained self-care hand functions, and could independently chew, swallow, and breathe. 10% of patients were graded from 7.0 to 7.5 EDSS points, 4% 6.0–5.5 points. A neuropsychological study found 33% of cases of pronounced cognitive impairment and 19% of moderate cognitive impairment.

A molecular genetic study in DMD patients found genetic polymorphism, with 82% of cases represented by deletion and duplication mutations in the dystrophin protein gene, as 12% by nonsense mutations. Mutations were detected in the DMD gene: most frequent mutations occurred from 43 to 50 exons; (n = 8) patients had mutations in the proximal end of the DMD gene in X chromosome (from 1 to 40 exon); and (n = 18) patients had mutations in the distal end from 41 to 79 exon. Analysis of mutation location in DMD gene showed that synthesis of dystrophin protein isoforms is impaired as follows: Dp260 (n = 18), Dp140 (n = 15), Dp116 (n = 1), and Dp71 (n = 1). 15 patients had the DMD gene mutation in exons that affect

synthesis of two isoforms (Dp260 and Dp140) (n = 15) and 1 patient, more than two isoforms (Dp260, Dp140, Dp116, and Dp71) (n = 1). Later, in order to clarify the role of mutation location in the DMD gene, we divided patients with confirmed mutations into two groups—those with mutation located in the proximal end of the DMD gene of X chromosome (from 1 to 40 exon) (n = 8) and those with mutation located the distal end from 41 to 70 exon (n = 16). We noticed that DMD patients with a distal end location of the DMD gene mutation had more pronounced cognitive impairment. However, we received no reliable data about the role of single isoforms of the dystrophin protein in development of cognitive impairment, as there had been insufficient clinical evidence.

In our study of DMD patients, we analyzed blood concentrations of NGF, BDNF, and CNTF growth factors by enzyme immunoassay. BDNF concentration in DMD patients was at a level comparable to the control group, and in some patients BDNF concentration was below the normal level of 21,500 pg/ml [18,650; 23,750] with the normal level of 24,454 [20,380; 29,640]. An enzyme-linked immunosorbent assay showed that serum NGF concentrations in DMD patients are 1,550 pg/ml [864; 1,901] higher than in the control group with 689 pg/ml [365; 987] ($p < 0.001$). CNTF concentration was higher in the blood plasma of DMD patients = 17.8 [12.4; 44.6] than in the control group, with the normal level of 14.9 [11.6; 21.6]. Comparing the concentrations of neurotrophins (NGF, BDNF, CNTF) in DMD patients, divided by the age, we found that patients under 18 showed a statistically significant ($p < 0.001$) excessive concentrations, in comparison with patients over 18. We received statistically significant ($p < 0.05$) results that NGF and BDNF levels are higher in the control group under 18 than in the group over 18. Patients with DMD with a mutation located in the distal end of the dystrophin protein gene (41 to 79 exon) have more pronounced cognitive impairments and a significantly decreased BDNF neurotrophin concentration in blood plasma ($p < 0.01$). DMD patients with cognitive impairment (n = 19) had decreased BDNF concentration of 23,670 [21,700; 30,720] pg/ml vs 32,700 [31,660; 33,750] pg/ml in DMD patients without cognitive impairment. We calculated the absolute risk of DMD with cognitive impairment by BDNF concentration, as well as odds ratios for the risk, and 95% confidence interval for the odds ratios. We divided the BDNF level into two intervals by the pattern of its distribution in the groups under study. **Table 1** shows the absolute risk of DMD with cognitive disorders and odds ratio of this risk, calculated by assessing relations between BDNF concentration and the course of DMD.

Having analyzed the distribution pattern of BDNF concentration in the groups, we identified two groups of patients—≤31,000 pg/ml and >31,000 pg/ml. The minimal risk of DMD with cognitive impairment made 20% (2 out of 10 patients)

BDNF concentration, pg./ml	DMD course without any cognitive disorders		DMD course with development of cognitive disorders		Odds ratio (OR)	95% confidence interval of the odds ratio (CI OR)
	Abs. number	%	Abs. number	%		
>31,000, n = 10	8	80	2	20	*	*
≤31,000, n = 20	5	25	15	75	12.0	1.9–76.4
Total, n = 30	64	42.7	86	57.3		

**The comparison group or the minimum predicted risk group.*

Table 1.
Assessment of the absolute risk of developing cognitive disorders in DMD depending on BDNF concentration.

in the group of patients with BDNF concentration over 31,000 pg/ml. Comparing with the group described above, patients with BDNF concentration less than 31,000 pg/ml had 75% risk of adverse course (15 of 20 patients); and statistically significant ($p < 0.001$) odds for cognitive disorders development were 10 times higher (odds ratio, 12,0; 95% confidence interval for odds ratios, 1.9–76.4).

The study proved that peptide neurotrophic regulation of the central nervous system has a complex nature in a current neurodegenerative process. We found that in DMD concentrations of neurotrophins are comparable to control data and tend to approach the lower normal range. Also, we noted a statistically significant difference in concentrations of neurotrophins (NGF and BDNF) in patients by age: in patients under 18, levels of neurotrophins are higher. This phenomenon can also be explained in terms of theory of neurotrophic neuromuscular interaction during ontogenesis, the key assumption of which is physiological role of neurotrophins synthesized by target cells (neurons and myocytes) to establish synaptic contact with a neuron.

We received controversial results in the laboratory study of patients' blood plasma in terms of content of various peptide substances involved in apoptosis and anti-apoptotic defense mechanisms. Analyzing laboratory results of levels of proteins involved in apoptosis (p53 protein, caspase 8, cytochrome C, Bcl2 protein) in the blood plasma of DMD patients, we found a significant increase in concentration of the proteins in such patients ($p < 0.01$) (**Table 2**).

It should be noted that many peptide substances show pathological activity in the nervous tissue. So, a high content of caspase 8, cytochrome C, p53 protein, and Bcl2 protein in blood of DMD patients indicates massive destruction of muscle tissue. Hyper-production of cytochrome C cannot be treated as a compensatory mechanism, as it is synthesized by mitochondria in the cell and can enter the bloodstream only due to cell destruction, similarly to the creatine phosphokinase enzyme in muscle pathology.

A study of DMD patients' blood plasma in an organotypic culture of nervous tissue showed that blood plasma of the patients has a weak effect on the growth of neurites of spinal ganglia, used as an experimental model. Area index in explants with blood plasma of DMD patients amounts to 105.0 [102.0; 108.0]%, which is less than the area index in the control explants 114.0 [113.0; 115.0] (**Figure 2**). The introduction of synthetic NGF (100 pg/ml) into the organotypic tissue culture with blood plasma of DMD patients increased the area index to 114.0 [111.0; 116.0]%. To explain that situation, we developed the theory of neurotrophic neuromuscular interaction during ontogenesis. In case of DMD, myocytes die, being unable to perform their physiological function not only as a contractile apparatus but also as a target cell. So, in primary muscle damage, synthesis of neurotrophins (NGF, BDNF) decreases.

Regulating proteins	DMD patients	The control group	Criterion Kruskal-Wallis, p
p53, U/ml	17.0 [4.0; 34.0]	0.0 [0.0; 0.4]	<0.001
Bcl2, ng/ml	46.0 [23.0; 87.7]	0.85 [0.0; 2.1]	<0.001
Caspase 8, ng/ml	0.18 [0.14; 0.31]	0.0 [0.0; 0.0]	<0.001
Cytochrome C, ng/ml	0.36 [0.0; 1.6]	0.0 [0.0; 0.0]	<0.001

*The results of non-parametric univariate analysis (Kruskal-Wallis ANOVA by ranks).

Table 2.
Concentrations of proteins involved in the process of apoptosis (p53, caspase 8, cytochrome C, and Bcl2 protein) in the blood plasma of patients of the studied groups, Me[Q_{25}; Q_{75}].

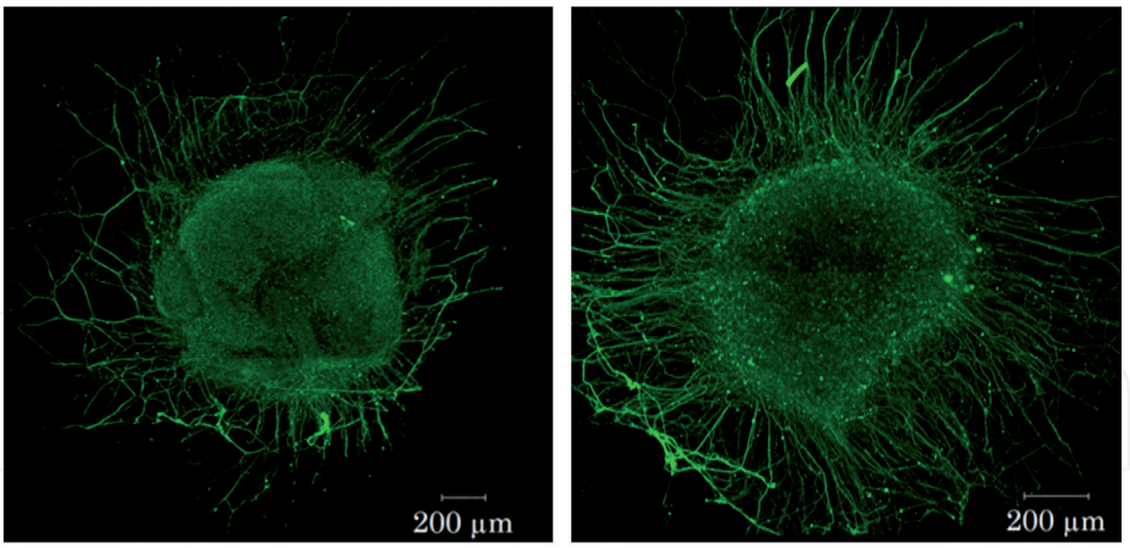

Figure 2.
(a, b) explant of the spinal ganglion of 10–12-day-old chicken embryo (3 days of cultivation) (microphotography). –(a) explant containing blood plasma of DMD patients, dilution (1:70); (b) explant containing blood plasma (control), dilution (1:70).

The clinical and neuropsychological study of DMD patients found cognitive impairments of varying severity in 33% of cases, and most of these patients had mutations of the DMD gene in exons 43–53, i.e., in the distal end. It is worth mentioning that among DMD patients with severe cognitive impairment (n = 4), three patients had a mutation affecting the Dp260 and Dp140 isoforms, and one patient had an impaired synthesis of Dp140, Dp260, Dp116, and Dp71 isoforms. These results prove that there are more pronounced cognitive impairment in DMD patients with a DMD gene mutation that affects synthesis of several DMD protein isoforms; however, we have insufficient clinical data to evaluate the reliability of these results. Analysis of the neurotrophin level in blood plasma in DMD patients shows a complex nature of peptide composition. We found that in the group of DMD patients with cognitive impairment, the concentration of BDNF neurotrophin significantly decreased; and the CNTF level was higher than normal. This may indicate an imbalanced neurotrophic regulation in the central nervous system structures; the imbalance manifests itself in a weakened BDNF effect on the nervous tissue, which results in decreasing rate of differentiation, synaptogenesis, and neuronal growth, on the one hand, and the increasing activity of glial cells, on the other. It causes a decreasing functional activity and dysfunction of neurons and leads to the development of cognitive impairment in DMD patients. However, we still cannot answer the question why it happens in DMD patients with a distal mutation in the dystrophin protein gene. Synthesis of BDNF neurotrophin is encoded on chromosome 11; however, Dp260 and Dp140 isoforms might be necessary for transport or transition from pro-BDNF to the active BDNF form. Perhaps, high concentrations of apoptosis proteins affect the p75 receptor and impair BDNF synthesis. Comparing our results with those of N. Doorenweerd (2014), who studied brain microstructure in DMD patients by quantitative magnetic resonance imaging, we can hypothesize about the role of this factor in cognitive deficiency development in DMD patients [29]. By the Doorenweerd study, DMD patients with an exon mutation of the Dp140(−) isoform and worse results of neuropsychological examination had a smaller brain volume and a smaller amount of gray matter than in the control group and the group of DMD patients with Dp140(+), who showed better results in the examination [30]. A decrease in brain volume may be associated

with a low level of BDNF neurotrophin, since we know that this factor stimulates growth and differentiation of brain neurons; however, vice versa supposition may also be valid: a small brain volume synthesizes a smaller amount of BDNF. So, we think that development of cognitive disorders in DMD patients is caused by a number of mechanisms, both of a genetic nature and based on neurotrophic regulation of the nervous system in DMD patients, as well as on secondary factors in terms of increasing activity of apoptosis proteins.

4. Conclusions

Thus, we detected a high content of apoptotic proteins (caspase 8, cytochrome C, p53 protein, and Bcl2 protein) and GNTF neurotrophin, and, at the same time, a reduced concentration of BDNF neurotrophin in blood plasma of DMD patients. In our opinion, this creates an intra-organic chemical imbalance and may serve as one of the factors leading to the development of cognitive impairment in DMD patients, together with such molecular-genetic factors as location of the mutation and impaired synthesis of dystrophin protein isoforms.

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