ALTERNATIVE APPROACHES OF CURING SPINAL MUSCLE ATROPHY

ALTERNATYWNE METODY LECZENIA RDZENIOWEGO ZANIKU MIĘŚNI

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Summary: Spinal muscle atrophy (SMA) is a autosomal recessive neurodegenerative disease. By the severity of manifested symptoms, four clinical types of SMA can be distinguished. Pathogenesis of the disorder is a result of mutation in SMN1 gene followed by motor neurons degeneration. SMN protein, which is a product of SMN1 gene expression, plays a key role in splicing. As a chaperone, it enables proper spliceosome formation. Splicing disruption mainly affects muscle neurons function. In the course of the disease, synaptic signal transmission is being deprived by the abnormalities in synaptic vesicles formation and their fusion with neurolemma. The consequences are serious and in severe stages include paralysis. On the cellular level, disorder can be characterised by Cajal bodies abnormalities.

Symptoms, including general weakness of spinal muscles, could be revealed by pharmacotherapy. The medication approved by FDA is Nusinersen. Although efficient, its colossal price prevents Nusinersen from being worldwide used. Scientists therefore focused on the development of alternative treatment methods. Three approaches are now extensively developing. First of them includes studies on the potential use of natural compounds in mitigation of SMA symptoms as well as finding a synthetic alternative to Nusinersen. Nowadays, particularly high hopes relate to Valproic acid – histone deacetylase inhibitor. Second approach links with usage of different than Nusinersen synthetic drugs, like Salbutamol (synthetic beta2-receptor agonist). Nonpharmacological approach is the use of genome therapy, which seems very promising, according to the latest clinical reports.

Keywords: SMA, valproic acid, Salbutamol, gene therapy

Streszczenie: Rdzeniowy zanik mięśni (SMA) to autosomalna recesywna choroba neurodegeneracyjna. Względem zaawansowania objawów, można wyróżnić cztery kliniczne typy choroby. Pato-

geneza choroby jest rezultatem mutacji w genie SMN1, poprzedzona degeneracją mięśni szkieletowych. Białko SMN, które jest produktem ekspresji genu SMN1, bierze udział w splicingu. Jako białko opiekuńcze, umożliwia utworzenie kompleksu spliceosomu. Zaburzenia składania mRNA, ma głównie wpływ na funkcję neuronów w ludzkim organizmie. W przebiegu choroby, przewodnictwo synaptyczne jest ograniczone poprzez nieprawidłowości w budowie i funkcji pęcherzyków synaptycznych, jak również ich fuzji z neurolemmą. Klinicznee konsekwencje są poważne i w ciężkich stadiach choroby powodują paraliż. Na poziomie morfologicznym, zaburzenie to może być scharakteryzowane dzięki widocznym nieprawidłowością w budowie ciałek Cajala.
Objawy, w tym ogólne osłabienie mięśni rdzeniowych, można uśmierzyć dzięki farmakoterapii. Lekiem do tego zaakceptowanym przez FDA jest Nusinersen. Pomimo jego skuteczności, jego wysoka cena zapobiega jego ogólnoświatowemu użyciu. Starania naukowców skupiają się zatem na poszukiwaniu alternatywnych metod leczenia. Obecnie szybko rozwijają się trzy podejścia. Pierwsze zawiera badania leków naturalnych w uśmierzaniu objawów choroby. Obecnie, duże nadzieje są wiązane z kwasem walproinowym – inhibitorze deacetylazy histonowej. Drugie podejście łączy się z użyciem innych niż Nusinersen leków syntetycznych, takich jak Salbutamol (syntetyczny agonista receptorów beta2-adrenergicznych). Niefarmakologicznym podejściem jest zastosowanie terapii genowej, której skuteczność potwierdzają najnowsze doniesienia kliniczne.

Słowa kluczowe: SMA, kwas walproinowy, Salbutamol, terapia genowa

INTRODUCTION

The spinal muscle atrophy (SMA) is a common disease, characterized by loss of lower motor neurons and progressive muscle wasting. The problem contributes also to the neuromuscular junctions, which are highly affected by the accumulation of neurofibrillary tangles in presynaptic neurons. The disorder is based on a defects of SMN genes, which codes a chaperon protein. These proteins play a crucial role in forming a spliceosome complex with Germin 2 and 8. The lack or defect of SMN gene causes the disturbances on the posttranscriptional level, which leads to neuromuscular disorders. The only registered by FDA drug for SMA treatment costs 75,000$ for an ampule [26].

The aim of this project was to gather information about alternative drugs, which could be useful in SMA treatment as well as to present the molecular basis of SMA. There have been analysed valproic acid (VPA), Salbutamol (Albuterol) in order to find weather these could be useful in therapy. Research made in 2005-2007 suggest, that VPA is not as effective as it was thought to be. Nowadays, the scientists are focused on Salbutamol, which strengthens the muscle tension and dilates bronchi. First results gives us high hopes of finding a low price, highly effective medicine for SMA treatment. The project focuses not only on the genetics and molecular basis of the disorder, but also on pharmacoeconomics of the problem.

GENETIC BASES OF SMA

SMA in 95% is caused by recessive modification in gene Survival Motor Neuron 1 (SMN1), which codes SMN protein [27]. Deletion or mutation appearing in gene 5q13.2 [17] causes to block the expression of the proper form of the protein. SMN protein appears in cytoplasm and cell nucleus takes part in forming snRNA, splicing of pre-mRNA and affects transport and translation of mRNA.

Concentration of the SMN1 mRNA level in fibroblast of people acted by SMA is usually ten times bigger than the level measured in control group [1].

SMN protein may also be produced from SMN2 gene, which is nearly identical in its structure to gene SMN1. It differs from SMN1 by one nucleotide (T instead of C). Only around 15% of copies mRNA built from SMN2 gene may result in creating proper SMN protein. Through this process, the damaged of SMN1 gene can be partially compensated by the SMN2 gene [8].

Genetic risk factors of SMA are mutations in SMN1 and SMN2. Normally, there are two forms of SMN gene – 1 and 2. When the mutation in SMN1 occurs, the multiplication of SMN2 form can compensate the loss of the first version of the gene. The multiplication can also recompense the defect in SMN2 gene by increasing the concentration of unstable and defected SMN2 protein, which in high concentration can act normally. Currently there are known substances, which are able to increase the level of SMN2 protein, and therefore reduce muscle atrophy symptoms in mice affected by SMA [2].

BIOCHEMICAL DEFECTS IN SMA

Spinal muscle atrophy (SMA) is a result of decrease of SMN protein in motoneurons. It is a chaperon, which enables the formation of snRNA complex with other protein components of the spliceosome [21]. With Germin 2 and 8 it allows on the formation of ribonucleoprotein spliceosome complex. The low level of SMN causes changes in snRNA, what leads to the abbreviation in gene expression on the basis of posttranscriptional modification [24]. Mice with SMN defect, exhibit low concentration of U12 and U4atac – spliceosome components. The research shows that introduction of proper SMN protein to the neurons with its knockout allows the neuronal growth and synapse formation[30]. Additional prof of the fact that SMA is caused by splicing disorders were the fluorescence microscopy pictures, which revealed that SMN is localised in Cajal bodies (CB). Interestingly defects of SMN do not affect the number or size of CB [24].

SMN is not the only defect protein in SMA. Another compound, which dysfunction is crucial for SMA is CHP1 and PLS3. CHP1 is an inhibitor of calcineurin, which is a Ca^{2+}-calmodulin dependent phosphatase, which catalyses the DMN1 dephosphorylation. That leads to the massive endocytosis in the presynaptic neuron in the synapse. Plasma membranes are used to form synaptic vesicles by filling them with neurotransmitter. The increased level of CHP1 makes it impossible, which results in the lack of synaptic vesicles. PLS3 is a calcium binding protein, which increases the level of F-actin, favouring the bulk endocytosis. PLS3 and CHP1 cooperate in the central nervous system development and synapse formation. The concentration of these two proteins increases between seventh and fourteenth week of development – soon after high levels of SMN form the first weeks decreases [6].

Other proteins, which have impact on the pathogenesis of SMA are FUS, GLE1 and Marten (MART3). FUS disturbs the transcription process by biding to the DNA and modulating transcription process. GLE1 acts on the transport of mRNA to cytoplasm. MART3 alters the RNA post-transcriptional processes of the mRNA [1].

Patients suffering from SMA tend to have two times diminished expression of GLE1 and MART3 genes and three times decreased expression of the FUS gene in comparison to control group. These proteins can be used in the future as a marker allowing to diagnose SMA in the foetus [1].

CLINICAL CHARACTERISTICS OF SMA

The spinal muscular atrophy is a disease caused by atrophy of neuronal cells in the anterior horn of the spinal cord. This leads to skeletal muscle movement impairment. Depending on the degree of atrophy level, this disease can be manifested by lowered speed and precision of muscle movement, through vanishing muscular reflexes, inability to walk, even to severe respiratory failure.

Spinal muscular atrophy divides into several main types, depending on the age of the patient and the level of muscular impairment. It is depended on the source. There are distinguished three to five types of this disease.

Type 0

This type come from Type 1 and is often named as type 1a. Type 0 is one of the most severe case of the SMA. It is characterized by very short lifetime of new--borns. Neonates with this form of SMA are unable to breathe by themselves, they are constantly ventilated by oxygen [4]. They live up to few weeks [8].

Type 1

Type 1 is often named as Werding-Hoffman disease. It is the most common type of the disease (around 60% of all SMA disorders) [27]. The SMA type 1

reveals up to sixth month of life. The lifetime usually does not exceed two years. Death is caused usually by insufficiency of the respiratory system. The Type 1 is appeared by many symptoms: decreased mobility of foetus, symmetrical hypotonia in new-borns, defects of the interventricular septum, reduced facial muscle mobility, weakness of intercostal muscles, which causes a paradoxical breath manifesting in rising of abdominal and chest collapse during inspiration. The notable sign is the child's inability to sit independently [13, 8].

Type 2

About 27% patients suffering from SMA have this type of disease[27]. This type of disorder reveals up to eighteenth month of life. The survivability is dependent on the severity of disease. The life expectancy is over two years and patients usually live up to adulthood. Death is caused most often by insufficiency of the respiratory system. The main symptom is symmetrical muscular weakness, which is primary manifested by vibrated of tongue and hands. Lower limbs have got decreased mobility in comparison to upper limbs. People with this type of SMA are able to sit independently but are not able to move independently [8, 14].

Type 3

The Type 3 is also named as Kugelberg-Welander disease. The SMA type 3 manifested after eighteenth month. Patients affected by this disorder usually live up to adulthood and old age. The main cause of dead is insufficiency of the respiratory system. The main symptom of the SMA type 3 is demonstrated by symmetrical muscular weakness, primary is manifested by trembled of tongue and hands and. weakened reflexes. The inferior mobility of the lower limbs compared to the upper limbs. The lack of reflexes is observed in the lower limbs. Patients are able to sit and walk at some point in their lives [8, 15]. The treatment with valproic acid improves the muscle movement of patients with this type of SMA [29].

Type 4

It is the mildest and the most uncommon type of disease, (less than 5% of cases of SMA) [8]. The illness is manifested between the ages of 20 to 60, usually around 35 years of age. The main symptoms are similar to SMA type 3 but they are milder [8, 16]. The treatment with valproic acid improves the muscle movement of patients with this type of SMA [29].

NEUROMUSCULAR JUNCTIONS

Neuromuscular junctions (NMJ) are also affected by SMA. In the developing foetus, acetylcholine receptors aggregate, forming pretzel-shaped structures, what enables the formation of the neuromuscular junctions [24]. The lack of the effective electric impulses from the spinal cord results in the degradation of the muscles.

NMJs can be divided in two groups. First consists of neurofilaments accumulation in presynaptic neuron and second of NMJ plate disorders. More severe courses are connected with the second group of the disorders [12].

Current research show that SMA acts each on various groups of muscles in different way [12], due to their different innervation. Muscles and their nerves can be divided into two groups by the tyrosine kinase MuSK and adaptor protein rapsin expression [9]. The first group – Fast synapsing (FaSyn), are more sensible to the neurofilaments accumulation than the second group – Delayed synapsing (DeSyn).

NMJs disorders lead to the decrease the half of amplitude of the electrochemical potential of the muscle sarcolemma. Unmodified are the simultaneous firing of the muscles, meaning that synapse transmission is defected. Moreover, the time of ions flow throughout the cell membrane is elongated, because of the extended time of the exchange of the foetal gamma-subunit of the acetylcholine receptor to epsilon-subunit. Epsilon subunit determines faster cations flow through the membrane. Disturbances in skeletal system are caused by slow exchange of foetal light chains of myosin to the mature ones[9].

THE ANALYSE OF EFFECTIVENESS OF USING DRUGS IN SMA

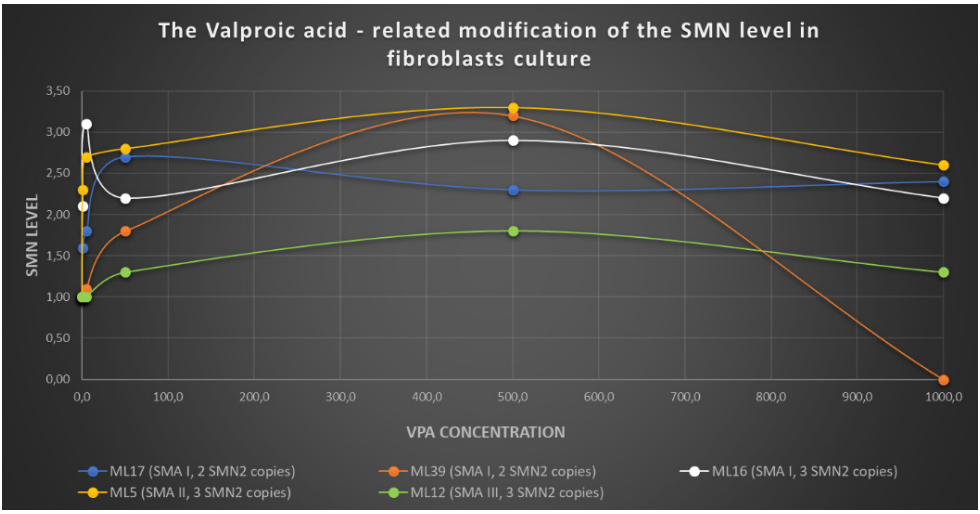
The only registered by FDA medicine for SMA treatment is SPINRAZA (Nusinersen), which [24] costs around $ 75,000 [26]. The rich price encourage scientists to look for alternative drugs. Two of the most explored promising compounds are Valproic acid and Albuterol.

The huge effort has been done in the field of finding a proper drug for SMA treatment. One of the potential medicines – Fluarizine has succeeded in tests on mouse, increasing the viability of SMA affected mice by 40%. Moreover, it has stopped the loose of the muscle mass and elevated the activity rate of the research rodents. The drawback of the treatment was, that there has been no strengthening of the muscles in comparison with the control group. Fluarizine decreases the level of PMZ protein, which results in the increase of low-mass RNA in the spinal cord – U1, U2, U4, U5, U6atac and U1 in the cerebellum [24].

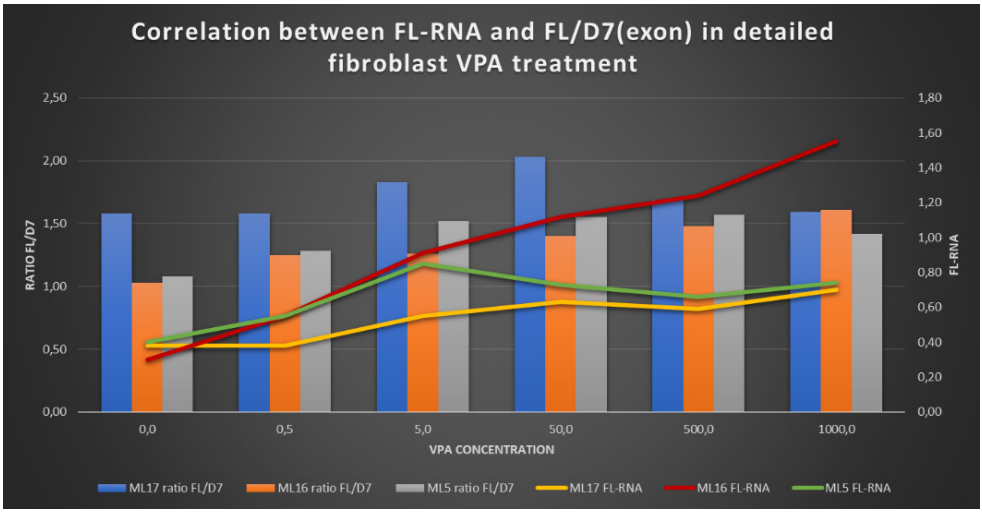
VALPROIC ACID

Valproic acid is a fatty acid with anticonvulsant and anti-manic properties that is widely used in the treatment of epileptic patients. The physiologic effect of VPA is used in the treatment of Epilepsy. The Valproic acid is a blocker of the sodium channel, which leads to the reduction of ionic potential of the central nervous system. In long-term of therapy, it can cause neurotoxicity. However, a full mechanism of its therapeutic actions is not well understood. Many research informs that Val-

proic acid has inhibition properties of histone deacetylase (HDAC) and increases SMN level in SMA patients by activation of SMN 2 (transcription and splicing correction of SMN2 exon 7). It is expected that VPA can be therapeutic agent of



GRAPH 1. The Valproic acid – related modification of the SMN level in fibroblasts culture (data from Rak et al. [22])
WYKRES 1. Kwas walproinowy – względna modyfikacja poziomu SMN w hodowli fibroblastów (dane z Rak et al. [22])



GRAPH 2. Correlation between FL-RNA and FL/D7(exon) in detailed fibroblasts VPA treatment (data from Rak et al. [22])
WYKRES 2. Korelacja między poziomem FL-RNA, a FL/D7(ekson) w leczeniu fibroblastów VPA (dane z Rak et al. [22])

SMA. In the paragraph, we present two studies evaluated by using the Modified Hammersmith Functional Motor Scale for SMA (MHFMS) [23].

In the experiment carried out in 2009 by Tiziano (et al.) fibroblast cultures were treated with 0.5-1000 mM of VPA for 16 h. The optimal period of treatment was established by a time covering a range of 12, 16, 24, 36 and 48 h. The cell culture was isolated from skin biopsies, carried out from five SMA patients [28, 22]: three with type I SMA, one with type II SMA and one with type III SMA. Every patients fulfilled the diagnostic criteria for SMA and transport homozygous deletions of SMN1. Fibroblast cultures were established according to standard protocols [22]. Results are presented on the graph 1 and 2.

Both types of research were made for the 16 h test optimized to 24, 36 and 48 h time covering range [22].

In another experiment, from January 2012 to March 2013, seven miscellaneous Japanese SMA patients were examined during 6 months of VPA treatment [23]. The group includes seven patients aged 2-42 years. The first patient (A) was a 34-year-old man who was treated with a placebo. The next one (B) was a 3-year-old woman. Patients C and D were accordingly a 23 years old man and a 30-year-old woman. The patient E was a 2 year and 10-month-old girl. Last two patients (F, G) were a 15-year-old girl and a 42 years old male with SMA type III. These results are presented on the table 1.

TABLE 1. VPA treatment results (data from Saito et al. [23])
TABELA 1. Rezultaty leczenia VPA (dane z Saito et al. [23])

Patient	Therapy month	VPA (mg)	VPA Concentration (μg/ml)	Ratio FL/D7-SMN	SMN (full-lenght)	Patient	Therapy month	VPA (mg)	VPA Concentration (μg/ml)	Ratio FL/D7-SMN	SMN (full-lenght)
A	0	-	-	0.28	1.19	D	6	400	70	0.98	2.25
B	0	-	-	0.50	1.02	E	0	-	-	0.67	2.59
B	1	400	50	1.17	5.47	E	1	25	13	0.95	3.18
B	3	400	45	0.95	3.27	E	3	75	25	0.88	4.27
B	6	400	42	2.05	1.61	E	6	100	34	0.58	0.68
C	0	-	-	0.20	2.82	F	0	-	-	1.29	1.01
C	1	200	39	0.36	4.78	F	1	200	18	0.88	1.37
C	3	400	62	0.78	2.96	F	3	400	26	1.15	1.24
C	6	400	75	0.39	3.09	G	0	-	-	0.55	1.12
D	0	-	-	0.73	3.61	G	1	200	47	0.60	1.31
D	1	200	35	0.60	2.96	G	3	400	50	0.40	0.73
D	3	200	34	0.77	2.51	G	6	400	43	0.53	1.77

Obtained results indicate, that Valproic acid (VPA), despite a large number of studies performed in 2005-2007, did not sufficiently confirm its effectiveness, although several individuals have seen some improvement. However, during the second experiment (Japanese patients), drastic SMN level changes could be an effect of false amount change between FL/ Δ 7-SMN. The data will balance out in next months, which is an example of short-term therapy effectiveness – probably caused by the placebo effect. Currently, VPA is rarely used due to unconfirmed and low efficacy (in long-term therapy) and some toxicity (including neurotoxicity), (2).

SALBUTAMOL (ALBUTEROL)

Albuterol is (±)-a1-[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol, which act as selective β2 receptor agonist. This medicine is used to prevent and treat wheezing and shortness of breath caused by asthma, chronic obstructive pulmonary disease. Albuterol belongs to a class of drugs known as bronchodilators. It works in the airways by opening breathing passages and relaxing muscles.

For now, in the literature are available two pilot studies evaluating the effects of albuterol in children with SMA. In both studies is observed significant improvement of muscle function [18, 7], as presented on table 2.

The significant advantage of using albuterol in the treatment of SMA is the relatively rare prevalence of adverse effects what is confirmed in presented studies. The double-blind clinical studies are required to involve albuterol to therapeutic protocols. The promising alternative is the salbutamol (albuterol), which systemize tonicity (β2 receptor agonist), and according to concrete studies, could improve endurance and make available low-cost treatment in some SMA patient cases. Due to the risk of side effects, it is used under the supervision of a physician.

TABLE 2. Results of Albuterol treatment (data from Pane et al. [18] and from Kinali et al. [7])
TABELA 2. Rezultaty leczenia Albuterolem (dane z Pane et al. [18] i z Kinali et al. [7])

SMA	Amount of subjects	Time of treatment	Results		
II	23	6-12 months	average increase between primary HFMS score and HFMS after 6 months of treatment	2.91 (1.97–3.85)	
				p<0.001	
			average increase between HFMS score after 6 and 12 months of treatment		0.91 (0.07–0.25)
					p=0.011
II	5	6 months	Myometry increase between baseline and after 3 months	21.6%, p=0.0001	
			Myometry increase between baseline and after 6 months	9.4%, p=0.017	
			MRC Scale increase between baseline and after 3 months	4.7%, p=0.004	
III	7		MRC Scale increase between baseline and after 6 months	3.4%, p=0.117	
			FVC increase between baseline and after 3 months	15%, p= 0.005	
			FVC increase between baseline and after 6 months	20%, p=0.030	

GENE THERAPY

Gene therapy is nowadays extensively studied as a potential method for SMA treatment [10]. Especially useful are modified adenoviruses, which could be applied at birth into the newborn [25]. Adenovirus, used in gene therapy could be specifically modified in order to integrate with DNA only in specific tissues.

Therefore SMN protein expression takes place only in central nervous system, omitting other proper tissues, which is undisputed advantage of the gene therapy [3].

After application of the virus, transported proper SMN gene, is being integrated with the hosts genome. Due to the specificity in integration with DNA in central nervous system, the expression of SMN gene occurs only in spinal cord [32]. The treatment effect of the therapy is the result of compensation of disrupted SMN gene by its proper form introduced by the virus. Curiously, the lack of SMN expression in muscles has no negative consequences, what proves that standalone central nervous system treatment is sufficient in SMA treatment [19].

Animal studies have proved the improved survival and motor function in response to genetical modification [19]. Animal trials prove, that the sooner adenovirus is being applied, the less chances of SMN to occur [5].

Application of gene therapy in SMN treatment is now in the clinical tests phase [31]. Although genes manipulation in human organism is highly controversial and leads to ethic problems, the results are incredibly promising. Mendell et al. have applied virus into fifteen patients, after 20 months after application all of them were alive (compared to 8% without treatment). Regrettably, SMA started to escalate in further life of the patients. However, gene therapy have not completely stopped the occurrence of SMA, it has significantly improved patients life quality [11].

CONCLUSIONS

However the antisense oligonucleotide – Nusinersen provide significant improvement in motor function and brings high hopes to patients affected by SMA there are several concerns about usage of this medication. Pharmacokinetic of this newly approved substance like way of administration, diffusion through brain blood barrier remains unknown like way of administration, diffusion through brain blood barrier[25]. Designed clinical trials did not give a conclusive answer about optimal time of administration, cause it is proved that in some of affected patients with SMA type 1 neromuscular impairments begins already in prenatal live[20]. The high price of nusinersen arouse ethical controversy about the equal allocation of treatment.

There is still wide array of promising methods which should be taken into consideration when discussing profitable way of SMA treatment. For instance, genome editing with adenovirus, which could become an causative therapy is actually under pre-clinical investigation.

Usage of albuterol could be an alternative to patients with developed neuromuscular impairment from mild to severe as an off-label adjuvant therapy. Some experimental studies indicate on possible stimulation of SMN2 full length transcript working protective in SMA affected patients. Argument in favour of salbutamol is low cost and relatively low presance of adverse effects. Pharmacokinetic of this substance is widely known down the years of usage in internal medicine.

Valproic acid (VPA), despite a large number of studies performed in 2005-2007, did not sufficiently confirm its effectiveness. However, several individuals have undergone improvement, which still gives hope about potential clinical application. Further research have to be done in the characterisation of VPA potential therapeutic properties.

Salbutamol and valproic acid should not be perceived as first-line treatment. Nonetheless, we highly believe that in cases of totally unethical and unfair distribution of modern treatment what could lead to advanced stages of disease Salbutamol and valproic acid as an supportive therapeutical method.

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