Are all new data referring to amyotrophic lateral sclerosis certain? Some doubts

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Progress in molecular investigations presents new data facilitating the recognition of pathogenic mechanisms of numerous nervous system diseases, among them amyotrophic lateral sclerosis (ALS). Molecular studies of ALS are mainly concentrated on genetic search, excitotoxicity and astrocytic participation, pathology of neurofilaments, apoptosis, trophic factors, and selective motoneurone degeneration.

In literature part of the results of these investigations are presented as certainty but some doubts exist. Problems of the role of the superoxide dismutase (SOD-1) mutation gene and free radical scavenging and selective vulnerability and death of motor neurone cells in ALS are mentioned.

key words: ALS, SOD-1 gene

GENETIC INVESTIGATIONS

In ALS, both in familial and recessive forms, gene mutations were observed within 7 chromosomes 9, 21, 2, 15, 1, 22, 8 [6, 16, 18]. Mutations of genes for heavy, medium and light subunits of neurofilaments (located on 4 chromosomes) were seen in animals in experimental model of ALS [2]. It is known that in transgenic mice with a mutation of gene for light subunit of neurofilament, a disease, clinically and morphologically similar to ALS, appears [17]. But particular attention is given to the mutation of the superoxide dismutase (SOD-1) gene and the role of this mutation in the pathogenesis of ALS. However, familial ALS occurs only in 10% of ALS cases, but among these cases SOD-1 gene mutation is observed in 21%. In sporadic cases and in population, mutation of this gene is shown in 3% and in 7% respectively. It should be accentuated that in familial ALS more than 60 different mutations of SOD-1 gene are known [8, 30].

The enzymatic function of SOD-1 is connected with conversion of the toxic superoxide radical to hydrogen peroxide and molecular oxygen, but the enzyme is ubiquitously expressed.

Disturbances in elimination of free radicals can cause (via oxidative stress) cellular damage. High expression of altered gene in CNS of transgenic mice was associated with age-related decrease of motor function and degeneration of motoneurones. Total levels of SOD-1 activity in mice tissues were normal [25]. Authors indicated that the neurodegeneration did not result from oxygen free radical scavenging.

This opinion is confirmed by the studies on experimental mice completely deficient in SOD-1. Animals develop normally and at 4 months of age spinal cord motoneurones reveal no signs of degeneration [24]. Estimation of various SOD-1 mutations in mice (with little effect of enzymatic activity) reveals the gain of function of these mutations [15]. Some authors [12, 32] considered the possibility that the gain of function may influence the disease by the formation of aggregates of toxic proteins. Investigations of the role of zinc (Zn) in copper/zinc (Cu/Zn) superoxide dismutase and metal-binding proteins were performed in SOD-1 gene mutation.
Replacement of Zn\(^{2+}\) with Cu\(^{2+}\) or Co\(^{2+}\) in some of these proteins changed the spectroscopic properties of these proteins and geometries of bindings of metal ions to the zinc sites. Mutation of SOD-1 may cause alteration of the zinc binding site and destabilisation of the protein [9, 20]. Loss of zinc in SOD-1 mutation leads to disturbances in oxidation of nitric oxide and motoneurone degeneration [13].

Incorporation of copper into SOD-1 is mediated through a specific metal carrier, copper chaperone for SOD-1 (CCS). Human CCS is encoded on chromosome 11 and expressed in all human tissue but it does not deliver copper to proteins in the nucleus, mitochondria or secretory pathway [8, 10]. Knockout of CCS (in yeast cell — Lys 7 Delta) leads to decrease of superoxide scavenging activity of SOD-1, but level of SOD-1 protein is normal [10].

Therefore, it is not clear how the SOD-1 mutation gene acts. Probably perturbations in free radical scavenging are not the leading factor but only one of many in ALS pathogenesis. It is also known that free radicals participate in numerous pathogenic mechanisms of various nervous system diseases as a result of metabolic cascade disturbances.

**SELECTIVE DEATH OF MOTONEURONES**

From the first description of ALS by Charcot and Joffrey [7] it has been known that loss of motoneurone cells within the spinal cord and brain stem is one of the characteristic features of the disease. Many years later selective vulnerability of motoneurones alpha was postulated [19]. As the result of mainly experimental investigations, the designation selective vulnerability or death was introduced. It is considered that the SOD-1 mutation gene leads to the toxic effect of free radicals on motoneurone cells [4, 11]. The Zn-deficient mutation of SOD-1 acts similarly [13]. In familial ALS, the toxicity of SOD-1 mutations led to motoneuronal death via an excitotoxic mechanism because the glutamate transporter human GLT-1 is inactivated [34]. In a model of slow toxicity in organotypic spinal cord culture inhibition of glutamate transport was manifested by degeneration of motoneurone cells [27]. It was also indicated that loss of astrogial glutamate transporter GLT-1 caused selective motor neurone degeneration via non-NMDA antagonist [28].

The GluR2 AMPA receptor subunit plays an important role in calcium permeability. In human motor neurones expression of GluR2 protein is low or absent [31, 33]. In ALS expression of GluR2mRNA is significantly lower only in anterior horn. Increase of calcium influx through AMPA receptor leads to neuronal vulnerability in ventral grey. Changes of GluR2mRNA “may be closely linked to the aetiology of ALS” [33].

However, in histopathological examination of severe ALS cases loss of not only motor neurone cells, but also interneurones and even astrocytes with very weak astrocystic reaction were observed [23]. *Nota bene*, SOD-1 immunoreactive inclusions within astrocytes were observed in transgenic mice [5].

Numerous populations of interneurones in the spinal ventral horn were found [1, 29]. A large group of these interneurones is composed of Renshaw’s cells, which recurrently inhibit motor neurone cells [14]. The excitability of Renshaw’s cells is set via the cortico-reticulo-spinal system [21, 26]. Degeneration of motoneurones may cause secondary degeneration and loss of Renshaw’s cells. But part of the interneurones are connected with Clarke’s column [29], therefore with the sensory system. Loss of interneuronal cells in ALS may indicate that not only motoneurone cells are selectively vulnerable in this disease.

Participation of the SOD-1 mutation gene in the selective vulnerability and death of motor neurone cells is also not clear. In normal conditions high levels of the enzyme were found in alpha motor neurones and the neocortex, but also in oculomotor nucleus neurones, substantia nigra, nucleus basalis and hippocampal sectors CA2, CA3, CA4 [3]. Severe oculomotor disturbances in ALS patients appear after a long time of disease duration when motor neurone cells of the spinal cord are absent. Nucleus basalis, substantia nigra and hippocampus are not damaged in ALS. Within the spinal cord intense SOD-1 reactivity, apart from motoneurones, was found in the sensory cells of substantia gelatinosa [22]. In ALS degeneration and loss of spinal ganglion cells are often observed [18]. It is also known that myelinated axons within dorsal roots are significantly reduced in number [19]. So, in ALS not only motoneurones, particularly of the spinal cord and brain stem, die, but also cells of the sensory system. The question arises whether the involvement of the sensory system in ALS is only a secondary process. Numerous morphological investigations indicate that morphological changes are considerably wider than a manifestation of clinical symptomatology.

It seems that the “selective death” of motoneurones limits the problem of ALS etiopathogenesis. It seems also that free radicals in the SOD-1 mutation gene as well as excitotoxicity (and other factors) are not etiological factors in ALS. They play a great role only in disease pathogenesis as metabolic cascade factors, which in various disease processes are observed. Motor neurone cells are probably more vulnerable than other cells of the nervous system, but they are not the only type of cells degenerating and disappearing in ALS.
REFERENCES


