



Research Article

Amyotrophic Lateral Sclerosis and Endogenous -Esogenous Toxicological Movens: New model to verify other Pharmacological Strategies

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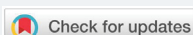
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Abstract

In 1874 J.M. Charcot was the first to describe ALS amyotrophic lateral sclerosis, a disease with a high non response therapy rate also to the actual therapy.

ALS is not clearly associate to only single etio-patogenetic movens but many process seem involved.

Also the strange geographic diffusion of different forms contribute to a complex syndromic pathology.

The introducing of new theories and approach can help to find more efficacy therapeutic strategies.

In this work the different neuronal damage movens and new therapeutic strategies are analyzed to produce a Unic global response to the pathologic process useful in next clinical application.

Genetic factors must be considered also added to environmental movens but also to the endogenous microenvironment of motoneuron involved.

A toxicological-biochemical-imunological approach can be useful tool to find new therapeutic strategies.

Or to improve local availability of pharmacological molecules.

Introduction

ALS is a chronic progressive neurodegenerative pathology involving motoneuron system (I and II, central and periferic or upper and lower motoneuron) with heavy symptomatology with progressive paralisy of voluntary muscle and since exitus.

Different form: primary, familiar 5-10%, et other involving young, adults, ereditary, sporadic, acquired.

In example see West Pacific foci, mutation SOD1 geography diffusion, sports related cases.

The primary damage is focused on perykarion of the neuron (and not in periferical axons like neuropaties).

As other neurodisease an intere system is involved (with similar physiology, biochemistry -metabolic), and etiology not clear.

Two main mechanism seems the pathogenetic moven s: a an increased toxicity by substancies (esogenous, endogenous, flogosis PGE2, m rna COX2, immune products, inclusions, excitotoxicity glutamate (see riluzole activity) calcium ion mediated, caspase activation, high affinity transporters like GLT-1 synaptic profile) or a weakness os some motoneuron system (or a combination of this) genetic profile, mutations (SOD 1 superoxide dismutase et other), enzymatic properties (mitochondrial functions).

Deficit in neurotrophyfactors et other.

Degeneration of motoneurons (cortex and spinal cord - pyramidal), damages in cortical - spinal tract.

Reactive gliosis reaction is common.

Various inclusion is observed in damaged neurons in various neurodegenerative pathology (alternative splicing activity is higher in brain tissue vs other tissue and this make possible to react vs different stimulus). Interaction between different RNA binding proteins with regulatory molecule in the pre mRNA s.

Pathological TDP-43 hyperfosforilated protein due by mutations (in sporadic ALS) differ from form in familiar disease.

Other mutation: FUSE/TLS, TARBDP, SOD1 D90 A and other.

And present aggregation role, activation of caspase, mitochondrial damages, alternated m rna axonal functionsand finally cellular death.

Other cell like astrocyte contribute to produce AMPA, microglia in produce TNF, metalloproteinase in this kind of cell damage.

Anterograde dying forward (glutamate mediated) starting from cortical region or dying back hypothesis due by deficit in neurotrophyfactors Periferically (neuromuscular site).

This last hypothesis due by the observation that the synaptic damage precede the motoneuron damage.

In some studies it was showed a toxic role of LCR ALS towards neurons culture.

Other esogenosu toxig agents: heavy metals (but not selective vs all neurons)? Cicas circinalis In GUAM cases? neuro latirism by Lathyrus sativus.

To be reported in example the incidence of ALS Death in some professional football players (and other sports) towards other causes (doping? Other causes?) and the case of west pacific foci (GUAM ISLE).

See also geographyc diffusion od SOD D90A, AD, AR and related minor or major aggressivity. (rapid or slow progression, few years vs 14 years in surviving according the different kind of SOD1 MUT.).

Form biomedical literature ALS in football players is related to the number of year of activity and seem related to the role (central players midfielder vs other like goalkeeper).

In first phases of disease is observed an increase in excitotoxicity, also oxidative stress is involved related to SOD mutations in some familiar forms. (Free radicals, then mitochondrial disfunctions, and progression with accumulation of catabolites like protein and neurofilaments inclusion).

Also cytoskeletal abnormalities in motoneuron axonx (very long) seem to be a weakness of the system. (neurofilament accumulation, altered axonal transport, deficit in neurotrophic factors).

Apoptosis of motoneuron is the final cell response to this toxic events.

Material and Methods

After observing some relevant in our opinion biomedical literature (pubmed) involved in pathogenesis and etiology of ALS we produce also a project of experimental approach to better clear this aspect (*in vitro*) and some drug delivery system strategy to improve tissue perfusion by pharmacological molecules or other substances.

Results

From literature

According Lyon M et al., "Amyotrophic lateral sclerosis is a severe debilitating pathology characterized by progressive degeneration of motor neurons. Charcot first described ALS in 1869¹; its pathogenesis remains unknown, and the effective treatments remain elusive. New paradigms must be investigated to understand the effectors of ALS, including inflammation, immune responses, and the body's response to stress and to the injury. We discuss the potential role of the immune system in ALS pathogenesis and critically review evidence from patient and animal studies. While immune system components may indeed play a role in ALS pathogenesis, results to indicate these cell, antibodies, and cytokines are localized to areas of early pathology are limited. We propose more focused studies that examine the role of the immune system with characterized pathogenesis to determine when, where and if immune and inflammatory processes are critical to disease progression, and thus worthy targets of intervention" [1].

Volk AE et al. written "ALS) is the most frequent motor neuron disease, affecting the upper and/or lower motor neurons. However, extramotor symptoms can also occur; cognitive deficits are present in more than 40% of patients and 7% of ALS patients develop frontotemporal dementia. There is no effective treatment for ALS and median survival is 3-4 years after onset. ALS sclerosis is a genetically heterogeneous disorder with monogenic forms as well as complex genetic etiology. Complex genetic risk factors are of minor interest for routine diagnostic testing or counseling of patients and their families. By contrast, a monogenic cause can be identified in 70% of familial and 10% of sporadic ALS cases. The most frequent genetic cause is a noncoding hexanucleotide repeat expansion in the *C9orf72* gene. High-throughput sequencing technologies in recent years have helped to identify additional monogenic and complex risk factors of ALS. Routine diagnostic testing should at least encompass the most frequently mutated disease genes (*C9orf72*, *SOD1*, *TDP-43*, *FUS*).. Caution is warranted as the *C9orf72* repeat expansion cannot be detected by routine sequencing technologies and testing by polymerase chain reaction is failure-prone. Predictive testing is possible in families in which a genetic cause has been identified, but the limitations of genetic testing (i. e., the problems of incomplete penetrance, variable expressivity and possible oligogenic inheritance) have to be explained to the patients and families" [2].

Di Pietro L et al. showed: “ALS is a fatal neurodegenerative disorder, no effective treatment to ameliorate the clinical manifestations is available today. The long-standing view of ALS as affecting only motor neurons has been challenged by the finding that the skeletal muscle plays an active role in the disease pathogenesis and can be a valuable target for therapeutic strategies. In recent times, non-coding RNAs, (microRNAs), have emerged as important molecules that play key roles in several cellular mechanisms involved in the etio-pathogenic mechanisms underlying various human disease. We summarize how the expression of some microRNAs is dysregulated in the skeletal muscle of ALS mouse models and patients. Shedding light on the mechanisms underlying microRNAs dysregulation in the skeletal muscle could clarify some of the processes involved in the pathogenesis of ALS and to identify new promising therapeutic targets in patients” [3].

Baskaran P et al.: “TDP-43-mediated protein-pathway is a key factor in the pathology of ALS. A potential underlying mechanism is dysregulation of the cytoskeleton. We investigate the effects of expressing TDP-43 wild-type, M337V, Q331K mutant isoforms on cytoskeletal integrity and function, using rat cortical neurons *in vitro*. We find that TDP-43 protein becomes mis-localised in axons over 24-72 hours in culture, with protein aggregation occurring at later time-points (140 hours). Quantitation of cell viability showed toxicity of both wild-type and mutant constructs which increased over time, especially of the Q331K mutant isoform. Analysis of the effects of TDP-43 on axonal integrity showed that TDP-43-transfected neurons had shorter axons than the control cells, and that growth cone sizes were smaller. Axonal transport dynamics were also impaired by transfection with TDP-43 constructs. These data show that TDP-43 mis-localisation into axons precedes cell death in cortical neurons, and that cytoskeletal structure and function is impaired by expression of either TDP-43 wild-type or mutant constructs *in vitro*. These suggest that dysregulation of cytoskeletal and neuronal integrity is an important mechanism for TDP-43-mediated proteinopathy” [4].

According Deng B et al.: “Myelination, degeneration and regeneration are implicated in crucial responses to injury in the peripheral nervous system. Considering the progression of ALS, we used the superoxide dismutase 1 (SOD1)-G93A transgenic mouse model of ALS to investigate the effects of mutant SOD1 on the peripheral nerves.

Changes in peripheral nerve morphology were analyzed in SOD1 mutant mice at various stages of the disease by toluidine blue staining and electron microscopy. Schwann cell proliferation and recruitment of inflammatory factors were detected by immunofluorescence staining and quantitative reverse transcription PCR and were compared between SOD1 mutant mice and control mice. Western blotting (WB) and TUNEL staining were used to investigate axonal damage and Schwann cell survival in the sciatic nerves of mice in both groups.

An analysis of the peripheral nervous system in SOD1-G93A mice revealed that: Schwann cells and axons in mutant mice underwent changes that were similar to those seen in the control mice during the early development of peripheral nerves. The peripheral nerves of SOD1-G93A mice developed progressive neuropathy, which presented as defects in axons and myelin, leading to difficulty in walking and reduced locomotor capacity at a late stage of the disease. Macrophages were recruited and accumulated, and nerve injury and a deficit in the blood-nerve barrier were observed. Proliferation and the inflammatory micro-environment were inhibited, which impaired the regeneration and remyelination of axons after crush injury in the SOD1-G93A mice.

The mutant human SOD1 protein induced axonal and myelin degeneration during the progression of ALS and participated in axon remyelination -regeneration in response to injury” [5].

Nguyen DKH et al. showed: “Age-dependent neurodegenerative diseases are

associated with a decline in protein quality control systems including autophagy. ALS is a motor neuron degenerative disease of complex etiology with increasing connections to other neuro-degenerative pathology such as frontotemporal dementia FD. Among the diverse genetic causes for ALS, a striking feature is the common connection to autophagy and its associated pathways. There is a recurring theme of protein misfolding as in other neurodegenerative pathology, but importantly there is a distinct common thread among ALS genes that connects them to the cascade of autophagy. But the roles of autophagy in ALS remain enigmatic and it is still unclear whether activation or inhibition of autophagy would be a reliable avenue to ameliorate the pathology. So The main evidence that links autophagy to different genetic forms of ALS is discussed" [6].

Mammana Se et al.: "In physiological conditions, different types of macrophages can be found within the central nervous system, microglia, meningeal macrophages, and perivascular (blood-brain barrier) and choroid plexus (blood-cerebrospinal fluid barrier) macrophages. Microglia and tissue-resident macrophages, blood-borne monocytes, have different origins, as the former derive from yolk sac erythromyeloid precursors and the latter from the fetal liver or bone marrow. Specific phenotypic patterns characterize each population. These cells function to maintain homeostasis and are directly involved in the development and resolution of neuroinflammatory Pathologic processes. Following inflammation, circulating monocytes can be recruited and enter the CNS, therefore contributing to brain pathology. These cell populations have now been identified as key players in CNS pathology, including autoimmune diseases, such as multiple sclerosis, and degenerative diseases, such as Amyotrophic Lateral Sclerosis and Alzheimer's disease. We review the evidence on the involvement of CNS macrophages in neuroinflammation and the advantages, pitfalls, and translational opportunities of pharmacological interventions targeting these heterogeneous cellular populations for the treatment of some brain diseases" [7].

According Shin JH et al. wrote: "While free radicals and inflammation constitute major routes of neuronal injury occurring in amyotrophic lateral sclerosis, but neither antioxidants nor non-steroidal anti-inflammatory drugs have shown significant efficacy in human clinical trials. We examined the possibility that concurrent blockade of free radicals and prostaglandin E(2) (PGE(2))-mediated inflammation might constitute a safe and effective therapeutic approach to the ALS disease. We have developed 2-hydroxy-5-[2-(4-trifluoromethylphenyl)-ethylaminobenzoic acid] (AAD-2004) as a derivative of aspirin. AAD-2004 completely removed free radicals at 50 nM as a potent spin-trapping molecule and inhibited microsomal PGE(2) synthase-1 (mPGES-1) activity in response to both lipopolysaccharide-treated BV2 cell with IC(50) of 230 nM and recombinant human mPGES-1 protein with IC(50) of 249 nM *in vitro*. In superoxide dismutase 1(G93A) transgenic mouse model of ALS, AAD-2004 blocked free radical production, PGE(2) formation, and microglial activation in the spinal cords. As consequence, AAD-2004 reduced auto-phagosome formation, axonopathy, and motor neuron degeneration, improving motor function and increasing life span. In these assays, AAD-2004 was superior to riluzole or ibuprofen. Gastric bleeding was not induced by AAD-2004 even at a dose 400-fold higher than that required to obtain a maximal therapeutic efficacy in superoxide dismutase 1(G93A). Targeting both mPGES-1-mediated PGE(2) and the free radicals may be a promising approach to reduce neuro-degeneration in ALS and possibly to be applied for other neurodegenerative diseases" [8].

According Okumura H: "Western Pacific amyotrophic lateral sclerosis in Guam, is a neurodegenerative disease with an high incidence among the indigenous population, Chamorros. To clarify the differences in the epidemiological and clinical features between Guam ALS and sporadic forms, the surveys were conducted in Guam for the periods from 1980 to 1989, in Rochester, MN, USA from 1952 to 1991 and in Hokkaido, Japan from 1980 to 1989. The crude incidence rate of Guam ALS was 7.5/100,000/year, which was much higher than the rates of sporadic ALS, 2.3/100,000/year

in Rochester and 0.6/100,000/year in Hokkaido, although it was markedly low as compared with that in the most frequent period between 1950-1960s. There was no remarkable change in the incidence rate either in Rochester or Hokkaido Island during the above study periods. The average age of onset of Guam ALS was 56, which was more than 10 years advancement occurring in the past 40 years, it was still younger than 68 and 58 in the sporadic ALS cases in Rochester and Hokkaido Island, respectively. The average duration of the illness in Guam ALS was 36 months, which was almost the same as those in Rochester (31 months) and Hokkaido (31 months). The changing ecology and socioeconomic conditions in the past 40 years in Guam might have contributed to the drastic reduction in the environmental risk factors. However, the incidence remains high during the past decade, which suggests their genetic predisposition to Guam ALS" [9].

Takeda T showed that: "Transactivation response DNA-binding protein 43 kDa (TDP-43) has been regarded as a major component of ubiquitin-positive/tau-negative inclusions of motor neurons and the frontotemporal cortices in amyotrophic lateral sclerosis and frontotemporal lobar degeneration (FTLD). Neurofibrillary tangles (NFT), an example of tau-pos inclusions, are biochemically and morphologically distinguished from TDP-43-positive inclusions, one of the pathological core features of Alzheimer disease (AD). Although ALS/FTLD and AD are distinct clinical entities, they can coexist in an individual patient. Whether concurrence of ALS/FTLD-TDP-43 and AD-tau is incidental is still controversial, because aging is a common risk factor for ALS/FTLD and AD development. is unclear whether the pathogenesis of ALS/FTLD is a direct causal link to tau accumulation. Recent studies suggested that AD etio-pathogenesis could cause the accumulation of TDP-43, while abnormal TDP-43 accumulation could also lead to abnormal tau- expression. Overlapping presence of TDP-43 and tau, when observed in a brain during autopsy, should attract attention, and should initiate the search for the pathological substrate for this abnormal protein accumulation. In addition to tau, other proteins including α -synuclein and amyloid β should be also taken into account as candidates for an interaction with TDP-43. Awareness of a possible comorbidity between TDP-43, tau and other proteins in patients with ALS/FTLD will be useful for our understanding of the influence of these proteins on the pathology development and its clinical manifestation" [10].

Pansarasa O et al.: "In 1993, Rosen et al. discovered that the gene encoding SOD1 has mutations in ALS these mutations are found in the exon regions, suggesting that their toxic effects are the consequence of protein dysfunction with an increase of the oxidative stress events. While a clear genetic picture has been delineated, a more complex scenario has been ascribed to the SOD1- protein. Some evidence sustains the hypothesis of an additionally toxic role for wild-type SOD1 (WT-SOD1) in the pathogenesis of sporadic ALS. On the other hand, our group identified a discrepancy among WT-SOD1 protein expression levels and mRNA in ALS sporadic patients, thus providing the hypothesis of a re-localization of the missing SOD1 in a different sub-cellular compartment, i.e., nucleus, or an aggregation/precipitation in the insoluble fraction. Our data also indicate an association between longer disease duration and higher amounts of soluble SOD1 within the nucleus, suggesting the possible defensive role of the protein in this compartment. We will attempt to resolve the ambivalent behavior of SOD1 in ALS disease and we will try to classify sporadic ALS patients according to a novel biological signature, SOD localization" [11].

According Oeckl P et al.: "To investigate the role of neuroinflammation in asymptomatic and symptomatic amyotrophic lateral sclerosis and frontotemporal dementia (FTD) mutation carriers.

The neuro-inflammatory markers chitotriosidase 1 (CHIT1), YKL-40 and glial fibrillary acidic protein (GFAP) were measured in cerebrospinal fluid (CSF) and blood samples from asymptomatic and symptomatic ALS/FTD mutation carriers, sporadic cases and controls by ELISA.

Our data indicate that neuro-inflammation is linked to the symptomatic phase of ALS/FTD and shows a similar pattern in sporadic and genetic cases. ALS and FTD are characterised by a different neuro-inflammatory profile, which might be one driver of the diverse presentations of the ALS/FTD syndrome” [12].

Patai R et al.: Showed: ALS, the most frequent motor neuron Pathology is characterized by progressive muscle weakness caused by the degeneration of the motor neurons in the spinal cord and motor cortex. According to the recent research, ALS is a complex syndrome which frequently involves symptoms of cognitive impairment. ALS cases can be interpreted in a clinico-pathological spectrum spanning from the classical ALS involving only the motor system to the fronto-temporal dementia FTD. The progression of the disease, manifested in the degeneration of the upper and lower motor neurons, is based on the same pathobiology. The main elements of the patho-mechanism, such as oxidative stress, excitotoxicity, immune-inflammatory processes and mitochondrial dysfunction are well described already, which operate in orchestrated way and amplify the deleterious effect of each other. It is assumed that calcium ions act as a catalyst in this interaction, hence each of the individual mechanisms has strong- positive reciprocal calcium- dependence thus may combine the individual pathological processes into a unified escalating mechanism of neuronal destruction. This review provides an overview of the role of calcium in connecting and amplifying the major mechanisms which lead to degeneration of the motor neurons in ALS” [13].

Manabe Y et al.: “The etio-pathology of amyotrophic lateral sclerosis remains unknown, an existence of neurotoxic substances in cerebrospinal fluid from ALS patients have been postulated. In order to better investigate a possible effect of CSF from ALS patients on cellular signaling in spinal neurons, we compared Fos-like immunoreactivity (Fos-LI) in organotypic- cultures of rat lumbar spinal cord after addition of CSF from ALS patients or another neurologic pathology. Fos-LI was normally present predominantly in dorsal horn neurons, whereas only a few ventral horn neurons were positive for Fos-LI. The number of

The Fos-LI + neurons significantly increased in dorsal horn with addition of CSF from ALS patients as well as glutamate at 100 microM. The increase was not observed with an addition of CSF from other neurologic pathology. The increase in Fos-LI + neurons in dorsal horn was reversed by a further supplement of MK801, an N-methyl-D-aspartate (NMDA) receptor antagonist, but not of CNQX, an alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate antagonist. These results indicate that there may be substances in CSF from ALS patients that stimulate Fos expression in certain populations of spinal neurons via the NMDA receptors” [14].

And according article Intra- Local Toxicology Aspect Time Related in Some Pathologic Conditions. “In toxicology usually are high considered the external- environmental factors but we think we must observe under toxicological methods also the inside intra/ extra cellular local- microenvironment (in paraphysiologic/pathologic conditions). In some disease the time is relevant added to local micro environment and inters cellular communication situations. We must consider an intra/ local toxicology aspect time related to better verify some pathologic process under a new light. In some time related local metabolic/catabolic-toxic status we can observe some cellular effect resulting in some final organ failure. The time involved in resolve some temporary gradients or the velocity involved in this process can be fundamental. The same effect related to too much rapid evolution / too slow reduction in balancing equilibrates physiologic systems. (The same the reduced effect showed in example in sports trained in SCD vs not trained activate platelet in trigger coronary artery spasms, amygdala temporal iper activation or other examples). We need to introduce more toxicological- methods in some disease in order to clear some relevant aspect In etiology diagnosis and therapy” [15].

And in article Brain and immune system: KURU disease a toxicological process was written observing also the different degenerative brain disease, with accumulation we can verify if exist an immune systems role. (AD, Parkinson disease, Lewy Body Dementia, Pick disease and other CNS amyloidosis) NEURODEGENERATIVE PROTEIN RELATED DISEASE (tauopathy), with brain accumulation and interference with many cognitive functions. Are there similarity in some neurodegenerative pathology like Tauopathy, alpha-synucleinopathy and CJD, prions disease? And related to other progressive dementias such as Alzheimer's and PD, ALS? (catabolic- cumulated immune toxic mediated process?) Is universally know that in example some plants produces alkaloids as bioproducts in their metabolism not having excretor apparatus as other animal organism. Can we consider waste of immune systems some accumulation substances' in some brain Pathology? (Materials that cannot leave from central nervous system: a global catabolic- finalistic process?) Observing this scientific - literature we can say that some neurologic disease can present common aspect: Accumulation of some metabolic- catabolic toxic substantia and related to the progression of disease and involved with immune system activation.

Chronic inflammatory process could be responsible for AD progression related to related immunity Response" [16].

Case A): "The field of free radical biology originated with the discovery of superoxide dismutase SOD in 1969. Over the last 5 decades, a plethora of research has been performed in species ranging from bacteria to mammals that has elucidated the molecular reaction, subcellular location, and specific isoforms of SOD. These enzymes have existed for billions -years, and may be some of the original enzymes found in primitive life. As life evolved over this expanse of time, these enzymes have taken on new and different functional- roles potentially in contrast to how they were originally derived.

The examination of the evolutionary history of these enzymes provides both an explanation and further inquiries into the modern-day role of SOD in physiology and pathology" [17].

According Naziroğlu M et al.: "In etiology of Alzheimer's disease, involvement of amyloid β (A β) plaque accumulation and oxidative stress in the brain have important roles. Several nano-particles such as titanium dioxide, silica dioxide, silver and zinc oxide have been experimentally using for treatment of neurological disease. In the last decade, there has been a great interest on combination of antioxidant compounds such as selenium (Se) and flavonoids with the oxidant nanoparticles in AD. We evaluated the data available on the physiological effects of oxidant and antioxidant nanoparticles. Areas covered: Oxidative nanoparticles decreased the activities of reactive oxygen species (ROS) scavenging enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase in the brain of rats and mice. Se-rich nanoparticles in small size (5-15 nm) depleted A β formation through decreasing ROS production. Reports on low levels of Se in blood and tissue samples and the low activities of the GSH-Px, catalase and SOD enzymes in AD patients and animal models support the proposed crucial role of oxidative stress in the pathogenesis of AD. Expert commentary: present literature suggests that Se-rich nanoparticles appeared to be a potential therapeutic compound for the treatment of AD "[18]

Adebayo OL et al.: Showed that: "Selenium (Se) and zinc (Zn) are trace -elements required for optimal brain functions. The role of Se and Zn against protein malnutrition induced oxidative- stress on mitochondrial antioxidants and electron transport chain (ETC) enzymes from rats' brain-CNS were investigated.

Normal protein (NP) and low protein (LP) rats were fed with diets containing 16% and 5% casein respectively for a period of 10 weeks. Then the rats were supplemented

with Se and Zn at a concentration of 0.15mgL(-1) and 227mgL(-1) in drinking water for 3weeks after which the rats were sacrificed.

The results obtained from the study showed significant ($p<0.05$) increase in lipid peroxidation (LPO), ROS production, oxidized glutathione (GSSG) levels and mitochondrial swelling and significant ($p<0.05$) reductions in catalase (CAT) and Mn-superoxide dismutase (Mn-SOD) activities, glutathione (GSH) levels, GSH/GSSG ratio and MTT reduction as a result of LP ingestion. The activities of mitochondrial ETC enzymes were also significantly inhibited in both the cortex and cerebellum of LP-fed rats. Supplementation with either Se or Zn restored the alterations in all the parameters.

The study showed that the Se and the Zn might be beneficial in protecting mitochondrial antioxidants and ETC enzymes against protein malnutrition induced oxidative- stress” [19].

Ravits JM et al. in 2009: “Heterogeneity of motor phenotypes is a clinically well-recognized fundamental aspect of amyotrophic lateral sclerosis and is determined by variability of 3 independent primary attributes: body region of onset; relative mix of upper motor neuron (UMN) and lower motor neuron (LMN) deficits; and rate of progression. Motor phenotypes are determined by the anatomy of the underlying the neuro-pathology and the common defining the elements underlying their heterogeneity are that motor neuron degeneration is fundamentally a focal- process and that it spreads contiguously through the 3-dimensional anatomy of the UMN and LMN levels, thus causing seemingly complex and varied clinical manifestations. This suggests motor-neuron degeneration in ALS is in actuality a very orderly and actively propagating process and that fundamental molecular- mechanisms may be uniform and their chief properties deduced. This also suggests opportunities for translational research to seek pathobiology directly in the less affected regions of the nervous system” [20].

McGeer PL et al.: “The ALS/PD complex of Guam is a long latency disease with a diverse phenotypic expression characteristic of classical ALS, PD and dementia. It is similar to a syndrome localized to the Kii Peninsula of Japan. There are as yet no identified pathological features that will clearly distinguish the Guam or Kii ALS/PDC syndrome from other degenerative neurological disorders. At present, ALS/PDC of Guam and the Kii Peninsula can be confirmed only by post-mortem examination. The most prominent pathological hallmark is the widespread occurrence of neurofibrillary tangles which express the same balance of 3R and 4R tau that is found in AD. They both show an increased prevalence of a peculiar retinal- disorder termed linear retinal pigmentary epitheliopathy. The disorders are both highly familial. Several environmental- factors have been proposed but no supportive evidence for an environmental / dietary factor has been founded. Genome searches have so far failed to identify causative genes although two single nuclear polymorphisms related to MAPT that increase the risk of the Guam -syndrome have been located. The 2 syndromes are clearly unique, and clues as to their causation could be beneficial in understanding the etiology of similar, but much more prevalent disorders in North America, Europe and Asia. Identification of the biomarkers for premortem- diagnosis would be helpful in management as well as in revealing the true etiology” [21].

AccordingH Muyderman et al.: “Mutant SOD1, ALS and mitochondria

Significant advances in understanding the mechanisms of pathology in ALS comes from studies using transgenic- rodents expressing mutant- forms of the human SOD1 gene that mimic the familial form of ALS (Gurney *et al.*, 1994; Nagai *et al.*, 2001). There are more than 140-150 known mutations in the SOD1 gene. Animals expressing mutant SOD1 typically display a phenotype that resembles ALS and demonstrate most of the histopathological / biochemical features and the symptoms of the human disease.

Pathology first appears in motor- neurons of the spinal- cord within 6-7weeks of birth and the first motor symptoms appear at 3-4 months of age resulting in a progressive paralysis similar to that found in humans (Chiu *et al.*, 1995; Julien and Kriz, 2006). Studies have demonstrated a role for mutant SOD1 in mitochondrial- dysfunction in ALS pathogenesis. Mutant SOD1 is often found as aggregates at the outer membrane of mitochondria in motor neurons of various mouse models and in fALS patients (Higgins *et al.*, 2003). It is believed that disruption of mitochondrial- function by the presence of misfolded protein- aggregates results in final mitochondrial damage, increased mitochondrial volume and excess superoxide production (Pasinelli *et al.*, 2004; Pickles *et al.*, 2013). Cells expressing some forms of mutant SOD1 undergo mitochondrial apoptotic signalling, mutant SOD1 transgenic animals overexpress proapoptotic proteins such as the BH3-only protein Bim and Bax while Bcl-2 and Bcl-Xl have been found to be decreased (Vukosavic *et al.*, 1999; 2000). Overexpressing Bcl-2 delays caspase activation in the mutant SOD1^{G93A} transgenic animal (Vukosavic *et al.*, 2000) and silencing Bim protein expression delays disease onset in other animal models of the disease. A similar anti-apoptotic effect is seen in cell culture models of mutant SOD1 ALS (Hetz *et al.*, 2007; Soo *et al.*, 2012). In mutant SOD1^{G85R}-expressing Neuro2a cells, Bim deletion leads to reduced Bax recruitment to mitochondria and decreased cytochrome C redistribution. As Bim is considered a direct link between endoplasmic reticulum stress and mitochondrial- apoptosis, these studies a clear pathway to cell death mediated by mutant SOD1 involving ER stress (Soo *et al.*, 2012). Mutant SOD1 could also damage mitochondria directly. Mitochondria containing mutant SOD1^{G93A}, but not wild-type SOD1, display changes in volume, aggregation, fragmentation and vacuolization (Higgins *et al.*, 2003; Sasaki *et al.*, 2004). Findings in human post-mortem or biopsy samples have reported abnormal mitochondria in cell bodies of motor neurons, proximal axons and in intramuscular nerves and skeletal muscle (Chung and Suh, 2002; Echaniz-Laguna *et al.*, 2002; Sasaki and Iwata, 2007). Such changes often precede disease onset in the mutant SOD1^{G93A} transgenic mouse and occurs before any other signs of motor neuron degeneration (Kong and Xu, 1998). These early changes are often followed by a substantial increase in mitochondrial vacuolization at the time of symptom onset (Kong and Xu, 1998; Bendotti *et al.*, 2001). Studies have shown that mutant SOD1^{G37R} binds directly to a voltage-dependent anion channel in the outer mitochondrial membrane and that this interaction inhibits channel conductance (Israelson *et al.*, 2010). Deletion of this channel results in decreased lifespan of the G37R mouse. Studies strongly suggest a direct link between mitochondrial viability and motor neuron degeneration and are further supported by other studies demonstrating sequential activation of caspase-1 and 3, with caspase-1 activation occurring before the onset of symptoms and caspase-3 activation being associated with later motor neuron loss (Pasinelli *et al.*, 2000). Consistent with these findings, treatment with a broad caspase inhibitor delays onset and slows disease progression in this transgenic mouse (Li *et al.*, 2000)" [22].

1965 CITATION: Lancet, 1(Mar 13), 593

From FDA poisonus plants database: The cycads of Guam. "Among the Chamorro, the indigenous population of Guam and the Mariana Islands, there is a very high incidence of amyotrophic lateral sclerosis and a parkinsonism dementia complex. The sclerosis was once thought to be inherited, but an environmental cause is suggested by the following facts: amyotrophic lateral sclerosis in Guam differs histologically from that found elsewhere; the condition is also commoner than expected in the non-indigenous population who have lived in Guam for ten years and more; and there are 2 other foci of high incidence in the Pacific on the Kii peninsula in Japan and in south west New Guinea. Other diseases such as diaphyseal aclasia, gout, and diabetes are unusually common in Guam; and preliminary studies show that the incidence of cirrhosis and cancer of the liver is higher than in the U S. Although the unusual figures

for these pathology may not be connected, they led to a study of the toxicology of the cycads which has provided enough material for a third conference in three years.¹ The Cycadaceae are palm like plants which have survived with little evolutionary change from the Mesozoic era. The seed of the cycad, *Cycas circinalis* L., is much used in Guam as a source of starch, especially in time of famine. The seed is shredded, soaked in water, dried, and ground into a flour. This is a lengthy process, and the indolent content themselves with halving the seeds before washing them. Effusions of cycad leaves are also used to promote the healing of wounds and ulcers. Attempts to reproduce the neurological disorders in animals by feeding meal or leaves from *C. circinalis* were successful only in a preliminary experiment using the rhesus monkey ²; but in the rat, mouse, and guineapig a hemorrhagic centrilobular necrosis of the liver developed. The water soluble toxin responsible for the liver damage, cycasin, has been identified as the glucoside of methyl-azoxy-methanol, and it is active only when a glucosidase is present to hydrolyse the toxin. Cycasin is not active when injected into rats and mice, nor when fed to germ free rats, because the action of the intestinal flora is necessary to liberate the active aglycone. Laqueur et al.³ showed that cycad meal induced a high yield of tumours of the liver and kidney of rats when incorporated in the diet for the duration of the experiment “.

According S.ZAREI et al.:“There is also geographical loci form of ALS where prevalence is 50–100 times higher in certain locations than in any other part of the world. These population include parts of Japan, Guam, Kii Peninsula of Japan, and South West New Guinea. Although this evidence is not concrete, it is believed that the increased incidence of ALS in these regions is due to environmental factors, specifically a neurotoxic nonprotein amino acid, β -methylamino-L-alanine (BMAA) in the seeds of the cycad *Cycas micronesica* produced by a symbiotic cyanobacteria in the roots of the cycad that are commonly found in these areas. It is hypothesized that patients in these regions who develop ALS have an inability in preventing BMAA accumulation.

More research is needed in South America to corroborate data for the rest of the continent, yet new studies show that the incidence of sporadic ALS in Uruguay is similar to those found within North America and Europe. Thus, it is important to continue epidemiologic studies of ALS in areas where little work has been done to identify vulnerable populations within Africa, Central America, and South America.

A possible relationship between ALS and sports participation has been proposed but not demonstrated. In a cohort study of Italian professional football players, a severe increase in the incidence of ALS was found. This study, conducted by the National Institute of Environmental Health Science, showed a correlation between head injuries in football players and an increased risk of ALS (odds ratio [OR] =3.2; 95% CI =1.2–8.1). Traumas to other parts of the body were not associated with increased risk” [23].

According: “Strategies for drug delivery to the central nervous system by systemic route”

Narayanan Kasinatha et al.: Delivery of a drug into the central nervous system (CNS) is considered difficult.

Most of the drugs discovered over the past decade are biological, which are high in molecular weight and polar in nature. The delivery of such drugs across the blood-brain barrier presents problems.

This review discusses some of the options available to reach the CNS by systemic route. The focus is mainly on the recent developments in systemic delivery of a drug to the CNS.

There are at least nine strategies that could be adopted to achieve the required drug concentration in the CNS.

The recent developments in drug delivery are very promising to deliver biological into the CNS.

Central nervous system (CNS)-related diseases and injuries are difficult to treat as most of the therapeutic agents are unable to cross blood–brain barrier (BBB) and blood–spinal cord barrier (BSCB). The last decade had witnessed an increase in biological-based therapeutic agents. Most of these substances are hydrophilic in nature. Therefore, it is imperative to design a suitable delivery system for such products so that they can cross BBB and reach the target sites within CNS.

BBB helps in maintaining a homeostatic condition within CNS. However, this provides a considerable hindrance while attempting to deliver drugs by systemic route. The drugs are unable to cross the BBB and hence have to be directly administered by invasive techniques. It was first demonstrated in the early part of the twentieth century by Ehrlich that all the tissues except the brain gets stained when injected intravenously with a dye (Finlay et al., 1996). Later in 1920s, it was shown that only those substances that are capable of entering cerebrospinal fluid (CSF) could affect CNS function.

After naming this selective drug permeability as “barriere hématoencéphalique” it was shown in 1960 that only the substances having high lipid solubility could enter CNS (Kroll & Neuwelt, 1998). Tightly packed endothelial cells with diverse receptors, transporters and efflux pumps help BBB in maintaining homeostatic condition within the brain (Begley & Brightman, 2003; Persidsky et al., 2006).

The BBB is tightly composed of endothelial capillaries that have less number of openings, less pinocytotic activities and more number of mitochondria compared to the endothelial cell junctions observed at the other sites in the body. These cells are further surrounded by astrocytic foot process and basal membrane. These cells along with pericytes form closely knitted junctions that are permeable only to lipid soluble substances (Selmaj, 1996). Presence of astrocyte foot process further provides a high integrity to the BBB.

However, solutes such as glucose, amino acids and nucleoside continuously enter CNS. These solutes are able to enter through the luminal and antiluminal portion of the BBB by carrier-mediated process (Pardridge et al., 1990b; Deeken & Loscher, 2007; Wolburg & Lippoldt, 2002). Permeability of these barriers is further influenced by the presence of astroglial cells, which regulates various signals involved in permeability of the BBB (Abbott et al., 2006).

Blood–cerebrospinal fluid barrier that starts at the choroid plexus forms an important barrier that regulates entry and exit of various substances to spinal cord. Although endothelial junctions of BCFB is not as tightly bound as that of BBB, the relative smaller surface area of this barrier compared to BBB, lower rate of diffusion and rapid rate of clearance effectively prevents entry of larger molecule and proteins and peptides (Bickel et al., 1993) [24].

Experimental project

ALS is a chronic disease and so is related to the progressive damage time related so is possible to think to a *in vitro* model that compare normal nerve tissue in first fase of disease with other normal nerve tissue not involved in this kind of pathology and observing if local microenvironment contribute in high way to the progression.

The same verify this condition in first phases and then advanced phases of disease (progression).

In this way is possible to confirm or not soluble factors involved.

The same also using the same liquid medium we can observe if present an intrinsic weakness of the nerve tissue affected vs normal tissue.

In this experimental project we can show even intrinsic weakness of neurons but also the activity of a soluble factor.

In example 4 *In vitro* experimental sample (animal model):

NEURONS SLA AFFECTED + Liquid medium of affected tissue

NEURON SLA AFFECTED + Normal liquid medium

NEURONS NOT SLA AFFECTED + liquid medium affected tissue

NEURONS NOT SLA AFFECTED + normal liquid medium

And other using nano drug delivery system to be added to pharmacological molecule to protect or deplete the microenvironment in some experimental test to verify any positive effect to avoid progression of damage. (in example the oxidative stress could be directed towards artificial “buffers” systems or other system to prevent the fibrillar deposition.

(In other field -discipline in example a zinc- coating prevent oxidation of some metals. So a Buffer system shift the phenomena from a material to another added to prevent oxidations).

Also with an idea to find strategies maybe novel drug delivery strategies to deliver the drugs or remedies that can trap drug vehicles close to the affected nervous tissue that through free radicals, phlogosis or other mechanism lead to the death of motor and long nerves' neural cells and therefore eventually lead to paralysis.

So what we are looking for this methods or a method that avoid systemic spreading the drug but somehow can get the drug just to spinal cord.

We speculate that this would be most likely in a parental dosage form, but no matter what we are looking for is something that can potentiate the efficiency of the drugs because of a higher access of the drug into the target local tissue without diluting the drug all over the body. This is stemming distribution of the drug through not only reducing potency but also increasing side effects which in the case of for instance corticosteroids therapy with increase the risk of damage to the immune system and therefore risk of infections and other.

One of our suggestions is for instance through spinal, radicular artery circle so we can site specifically get into the capillary bed of that nerves, through arteries that irrigate those nerves. Luckily this type of disease is can start in specific tracts of spinal cord so to speak thoracic, lumbar or other parts of the vertebral column. The idea to reach these nerves through incoming bloodstream obviously arteries that irrigate these nerves is our the main ideal goal, but we don't exclude possibilities of some sort of drug delivery systems or even colloidal particles such as nanoparticles that can be stopped in those capillary beds, like for example magnetic nanoparticles or other ways such as conjugated with monoclonal antibodies or maybe some other techniques which we don't know of.

Regardless of technology, our main goal is to have the drug accessing only the nervous tissue involved and no other organs or tissues or to limit the distribution of drug molecules or drug vehicles in that specific capillary beds, so site specifically the nerve can be treated in some sort of locoregional delivery fashion.

Other strategies or technologies could be for example and non-invasive or minimally invasive implantation of a polymeric paste or some sort of stent or device close to reach the nervous tissue involved.

Again, the goal is to limit the delivery of the drug, to maximize the drug levels in proximity of the nervous tissue with a plan of reducing side effects in other sites, potentiate and extend the delivery and backup the presence of the drug, preventing its levels from falling after every single administration.

The problem with theoretic¹ CSF implants model is that they have to have the same chemistry of the CSF fluid. It means they have to have the same viscosity or you have to be isotonic with the CSF also the same pH, and other composition otherwise did the chemistry of the injected in intraCSF is different than the CSF itself is probably would leave some damage to the central nervous system. One example there is an interest CSF vial with antitumoral drug called Methotrexate, which because of having similar CSF physicochemical conditions it has been successfully tolerated.

Nanoparticle coated with 400 Dalton (or other waight) PEG could be acting as biovectorized artificial protein-free low-density lipoproteins and hence not only bypass reticuloendothelial system phagocytosis but also cross the blood-brain barrier BBB. These are called “long circulating colloidal drug carriers” because they resist cleansing or filtering by reticuloendothelial system RES located in kidney, spleen, lymph nodes, lung, and bone marrow. So far they use solid lipid nanoparticles, including those that contain gelatin and liposomes, it means stealth SLN and liposomes. Now solid lipid nanoparticles are also mentioned for SUV or small unilamellar vesicles which is a 100 nm liposomes, which are produced by Extrusion of a mixture of phospholipids drug and water.

Of course the best is to avoid a complex surgery combined with a depuration strategy, a technology used for detoxification of certain poisons, but if we can't site specifically target the drug to that specific anatomical location probably that's the only option left to prevent further progress of the disease.

Upasana Sharma et al.: “This paper represents review covering various aspects of the Central Nervous System targeted drug delivery using polymeric nanoparticles with respect to targeted drug delivery to CNS and its need, barriers of CNS, which prevents the entry of therapeutics, various strategies used to manipulate drugs to cross the blood brain, and blood cerebral spinal fluid barriers.

CNS disorders are the major worldwide public health problem. The CNS is one of the most delicate and sensitive micro environments of the body. It is protected by the blood-brain barrier (BBB) regulating its homeostasis.

Many strategies are currently being required to enhance the delivery of drugs and molecules across the BBB.

Generally the size ranges of nanoparticle which are from 10 to 1000 nm (50–300 nm generally). The use of small particles as drug carriers for targeted delivery has been studied over a long time.

Polymeric Nanoparticles have been shown to be promising carrier for the delivery of drugs in the CNS because of their potential both in encapsulating drugs, hence it protect them from excretion and metabolism, and in delivering active agents across the BBB without producing any damage to the barrier. Various polymers have been used and different strategies like surface modification have been used to increase the retention time of nanoparticles.

The use of biodegradable polymeric nanoparticles (NPs) for controlled drug-delivery has shown significant therapeutic potential. The development of other useful polymeric NPs to deliver a spectrum of chemotherapeutic-diagnostic-imaging agents for various applications. Polymeric nanoparticles signify one of the most motivating challenges for the technical world being investigated as drug delivery systems for effective complete-local delivery of therapeutics molecule to the central nervous system. Nnanoparticulate systems have gained increasing interest within therapeutic strategy.

Polymer nanoparticles are the particles of less than 1 μm diameter that are prepared from natural or synthetic polymers. Nanoparticles can deliver a wide range of drugs to different areas of the body for sustained periods of time.

The smaller size of nano-particles is integral for the systemic circulation. Composed of synthetic / semi-synthetic polymers.

Biodegradable polymeric nanoparticles like polylactic acid (PLA), polyglycolic acid (PGA), poly(lactide glycolide) (PLGA), and polymethyl methacrylate (PMMA) phospholipids hydrophobic core.

They show a good potential for surface modification via chemical transformations, and provide good pharmacokinetic control, and are suitable for the entrapment and delivery of a wide range of therapeutic agents and molecules.

This polymeric coating is thought to reduce immunogenicity, and limit the phagocytosis of nanoparticles by the reticuloendothelial system, resulting in increased blood levels of drug in organs such as the brain, intestines, and kidneys.

They may be formulated to encapsulate several classes of therapeutic agents and molecules including, but not limited to, low molecular weight compounds. Natural polymers like proteins or polysaccharides have not been widely used for this purpose since they vary in purity, and also require cross linking that could denature the enclosed drug" [25].

According P.KURY et al. "HERVs, retroviral sequences integrated into the genome during evolution, are now known to represent 8% of the global human genome.

These were recently shown to comprise copies that retain potential to express retroviral proteins or particles, and can be abnormally expressed in autoimmune/ neurodegenerative/chronic inflammatory diseases/ and cancer.

Environmental factors such as specific viral infections were shown to potentially activate HERVs under tissue-specific and temporal conditions.

Of several pathology in which abnormal activation and expression of HERV proteins have been reported, studies over recent decades have led to a proof of concept that HERVs play a key role in the pathogenesis of MS and ALS.

HERV-W and HERV-K Env proteins induce pathogenic effects *in vitro* and *in vivo* that are relevant to the pathognomonic features of these diseases.

These endogenous retroviruses are potential novel therapeutic targets that are now being addressed with innovative therapeutic strategies in clinical trials" [26].

L R Hanson et al. " delivery provides a practical, non-invasive method of by-passing the blood-brain barrier (BBB) to deliver therapeutic molecules to the brain and spinal cord. This technology allows drugs that do not cross the BBB to be delivered to the CNS within minutes. It also directly delivers drugs that do cross the BBB to the brain, eliminating the need for systemic administration and its potential side effects. This is possible because of the unique connections that the olfactory and trigeminal nerves provide between the brain and external environment. Intranasal delivery does not necessarily require any modification to therapeutic molecules. Variety of therapeutics, including both small molecules and macro-molecules, can be targeted to the olfactory system and connected memory areas affected by Alzheimer's disease.

Using the intranasal delivery system, researchers have reversed neurodegeneration and rescued memory in a transgenic mouse model of A. D.

Intranasal insulin-like growth factor-I, deferoxamine, and erythropoietin have been shown to protect the brain against stroke in animal experimental models. Intranasal delivery has been used to target the neuroprotective peptide NAP to the brain to treat

neurodegeneration. Intranasal fibroblast growth factor-2 and epidermal growth factor have been shown to stimulate neurogenesis in the adult animals. Intranasal insulin improves memory, attention, and functioning in patients with A.D. or mild cognitive impairment, and even improves memory and mood in normal adult humans. This new method of delivery can revolutionize the treatment of Alzheimer's disease, stroke, and other brain disorders.

The use of intranasal (IN) administration to target therapeutics to the CNS has many benefits in the treatment of neurologic disorders. The blood-brain barrier (BBB) restricts the use of numerous therapeutic agents that have been developed to treat memory loss and neurodegeneration because it limits CNS penetration, depending on drug size or charge.

Invasive methods of administration (for instance intra-cerebroventricular) have been used to overcome the BB but these methods are not practical for use in humans for several reasons, including convenience, safety, and cost.

Direct delivery of therapeutics from the nasal cavity into the CNS (IN delivery) bypasses the BBB and provides an alternative way to invasive methods of pharmacological drug administration.

Non-invasive IN delivery targets therapeutics to the CNS, reducing the systemic exposure and side effects; this can be advantageous for delivery of many CNS pharmacological therapeutics, including those that can cross the BBB upon systemic administration.

CNS therapeutics do not necessarily need to be modified for IN delivery, and delivery of therapeutics to the CNS is rapid, within minutes. The IN delivery method was first developed by Frey in 1989 for targeting neurotrophic factors (in ex nerve growth factor and fibroblast growth factor-2) to the CNS.

Intra-nasally administered therapeutics reach the CNS via the olfactory and trigeminal neural pathways. Both nerves innervate the nasal cavity, providing a direct connection with the CNS.

Direct delivery of therapeutics from the nose to the brain was initially attributed to the olfactory pathway. More recently, the contribution by the trigeminal pathway to IN delivery to the CNS has also been recognized, especially to caudal brain regions and the spinal cord.

Extracellular delivery, rather than axonal transport, is strongly indicated by the short time frame (≤ 10 minutes) observed for IN therapeutics to reach the brain from the nasal mucosa. Mechanisms of transport may involve bulk flow and diffusion within perineuronal channels, perivascular spaces, or lymphatic channels directly connected to brain tissue or cerebrospinal fluid" [27].

According J. LIU et al. "Effective therapy for ALS is still in its infancy, even after years of intense investigation with chemical compounds and cell-based treatment. The pathogenesis involved in MN death in ALS is complex, and neuroinflammation has been accepted as a key contributor to MN degeneration and disease progression. However, a big challenge regarding the development of new therapies for ALS patients is the failure to translate positive preclinical results into successful clinical practice. Several issues should be taken into consideration when designing therapeutic strategies targeting neuroinflammation in ALS. First, most preclinical ALS studies invariably employ the mSOD1 transgenic rodents (generally SOD1^{G93A} mice), which is a limited disease condition related to a small subgroup of ALS patients with SOD1 gene mutation. Therefore, transgenic mouse models with different gene mutations (e.g., TDP-43 and C9orf72) should be employed in preclinical studies to find out whether

they share common mechanisms involved in neuroinflammation. Second, therapeutic strategies in transgenic animals are usually applied during presymptomatic or slowly progressive disease stage. Despite promising treatment outcomes in these preclinical studies, they cannot be translated into patients because most ALS patients are identified and diagnosed during the late and rapidly progressive phase. Third, the inflammatory environment of ALS varies with disease progression, and involves both neurotoxic and neuroprotective aspects. Thus, specific therapeutic timing may influence the pathogenic target and choice of drugs. Fourth, the heterogeneity of patients may also contribute to the failed translation of therapeutic effects from homogeneous transgenic animals to ALS patients. In addition, one should consider promoting anti-inflammatory and neuroprotective properties of immune cells, instead of simply and completely suppressing inflammatory and immune responses to achieve precise and personalized treatment for ALS patients." [28].

An interesting example comes from other different field but that can be useful to our experimental project: cars body parts protection in the beginning of the 20th century. When steel was rusting chemist-oriented scientists came up with an idea to include a small portion of zinc, as the coding these steel parts, as Zinc has more tendency to oxidize than iron, so the oxidation would be diverted to zinc, instead of iron. Could we take the same principle and apply to some sort of drug or other artificials system, which can do the same to the nerves? So to speak we want something to stop the radical chain of the damage inflicted upon the defenseless nerve so something else takes the damage away from the nerve. The pharmaceutical scientists suggested the use of antioxidants such as Vitamin C, vit E and other, but showing unsatisfiable response. In other words may be there is no enough vitamin C or other antioxidants sustained at the target?

So since after each administration, the drug levels at the target fall immediately or in some cases in a short spans of time would a sustained release in a locoregional fashion can recompense this process of loss of activity? That's assuming that the troublemakers are free radicals. But regardless, once we can find a technology that can deliver site-specifically the drug to the tissue around the nerve around the part of the body that specific location in which say for example lumbar the damage is happening, of course with an extended release feature, instead of antioxidants, we may use other drugs, including corticosteroids or other molecule ?. So we believe there are two problems: one is that we don't exactly know with what mechanism this phlogosis damage is happening, but more importantly we certainly have also a pharmacokinetic and biodistribution problem.

This is for the case of peripheral damages. But we also have some cases in which the damage is happening at the central nervous system, specifically the cortex and the spinal cord. What are we going to do with those cases? Do we still have the option of implants which requires surgery? How it will look for something that can deliver the drug buy systemic administration? By maybe vehicles that can cross the blood-brain barrier? Do we have such possibilities? Obviously there are some drug carriers, colloidal submicron drug carriers, that are able to cross the blood-brain barrier to break through the BBB. Something like natural or artificial nutrient carriers in the blood, such as transferrin, or a sort of protein free artificial low density lipoprotein system, which looks like natural carriers for aliments or nutrients for the brain, a trojan horse that can have the password at the gate. Can we use such technology whether are colloidal particles, nanoparticles, PEG coated liposomes, you name it, anything that can site specifically deliver the drug to cortex or certain parts of spine? Do we have to refuge to intra cerebrospinal fluid injections?

(We know some drugs such in example Nusinersen intratecal indicated for the treatment of spinal muscular atrophy (SMA), is a survival motor neuron-2 (SMN2)-directed antisense oligonucleotide).

Discussion

Related the analysis of the reported literature (and to be verified with the experimental project hypothesis) we can say that In this kind of disease the different theory seems to show an increased toxicological effect in the endogenous neuronal microenvironment (the weakness of some neurons vs other can enhance the Velocity of the damage).

Different signals can start the apoptotic process in a common final effect.

Conclusion

Related the body region of onset, mix of upper and lower motoneuron deficits and rate of progression

The endogenous neuro microenvironment in determinate genetic profile is heavily involved with the neuronal damages and strategies

That can control or modify it can be useful in preventing progression of some neuro chronic degenerative disease.

An esogenous or endogenous toxicology approach (similar to an antidotes approach or depurative strategies) added to the best new pharmaceutical instrument can be a way to be run to protect the motoneuron from a poison like process.

The cell death due by apoptosis (free radicals, excitotoxicity, flogosis, immune reactions, toxic exogenous substancies and other can be avoided or reduced introducing new depurative strategies (against TOXIC -X Or dangerous micro- or local environment) or other Teqniques to shift the oxidative damage from the motoneuron to other substancies (or other physich procedure, medical devices and other artificial implants to improve global activity).

If considered like VECTORS in Physical science the 2 vectors: intrinsic neuronal weakness and the esogenous endogenous toxicologic substancies moves in the same direction: a moto neuron damage.

Related to this conclusion new pharmacological strategies or high improving in local availability of therapeutic substaties can improve clinical outcomes by clinicians. (better efficiency in pharmacokinetics, BEE pass level, persistence of action, low local toxicity, and other properties, medical devices uses, innovative nano drug delivery systems, alternative way of subministration).

A Medicinal chemistry- pharmaceutical and toxicological approach can be the right instrument to be added to the actual therapeutic scenario of this neurodegenerative disease (better pharmacokinetics in BEE pass can be relevant also in other field like oncology- ematology).

Clarifications

This work is produced with a toxicological-medicinal chemistry – biochemistry approach.

And produced without any diagnostic or therapeutic intent.

Only to produce new research hypotesys.

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