(Pixabay)

FULLERENES

Do they have therapeutic potential in Alzheimer's disease?

The potential of fullerenes to interact with Aβ and to exert an effect on the diseased AD brain in experimental animals is particularly important considering that extracellular irregular protein aggregates are also present in the specific age-related form of neurodegeneration defined as mild cognitive impairment (MCI). The potential of 3HFWC to slow down protein aggregation and decrease the speed of subsequent neurodegeneration is intriguing. These findings substantially broaden the potential therapeutic value of 3HFWC, particularly as a general prophylactic in aging population.

Sanja Ivkovic
Milka Perovic
Đuro Koruga
Selma Kanazir

Alzheimer's disease - is it an epidemic?
Alzheimer's disease (AD) is a debilitating disease. It is characterized with the irreversible and progressive brain deterioration, slow destruction of memory and thinking skills and, finally, the ability to carry out the simplest life tasks. It is currently affecting about 50 million people worldwide and this number is estimated to increase 62% by 2030. Nowadays, with the substantial advances in modern medicine, our life-span has been significantly increased (Figure 1). The United Nations estimates a global average life expectancy of 72.6 years for 2019 – the global average today is higher than in any country back in 1950. Paradoxically, advanced age is the single most major risk for AD with the prevalence doubling every 5 years between the ages of 65 and 95 years and increasing from 2% at 65 years of age to 40% at over 85 years of age. The elderly experience minor changes in memory and thinking capacities but these changes are not severe enough to affect daily living and functioning. Most importantly, the ability to live independently is not affected.

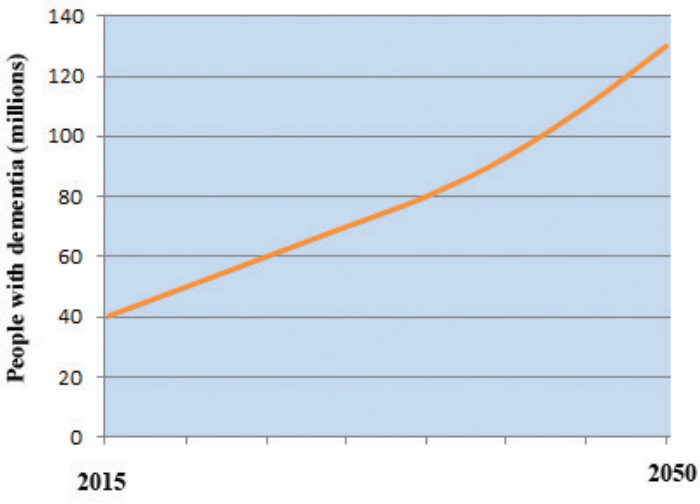


Figure 1.

However, AD patients are being gradually stripped of their ability to make decisions and participate in everyday life and as the illness progresses they become completely dependent on help from family members and other caregivers. The typical first signs of AD are memory problems, although initial symptoms may vary from person to person. A decline in other aspects of thinking, such as finding the right words, vision/spatial issues, and impaired reasoning or judgment, may also signal the very early stages of Alzheimer's disease. These results in inability to perform everyday things like driving a car, cooking a meal, or paying bills. People with Alzheimer's disease often ask the same questions over and over, they get lost easily, lose things or put them in odd places, and find even simple things confusing. As the disease progresses, some people become worried, angry, or violent. Although memory loss and dementia, in general, are progressive and irreversible, the rate of progression of these symptoms is highly variable and impossible to predict.

The complete helplessness of often otherwise healthy AD patients is devastating and heartbreaking. On the other hand, as the aging population progressively increases in modern society, the high incidence of AD among the elderly poses a substantial socio-economic burden to patients, caregivers, and the healthcare system. The estimated total healthcare costs for the treatment of Alzheimer's disease in 2020 in the USA is $305 billion, with the cost expected to increase to more than $1 trillion as the population ages. Most of the direct costs of care for Alzheimer's disease are attributed to skilled nursing care, home healthcare, and hospice care. Indirect costs of care, including quality of life and informal caregiving, are likely underestimated and are associated with significant negative societal and personal burdens.

Alzheimer's disease was discovered in 1906
The disease was discovered in 1906 by Dr. Alois Alzheimer, a German psychiatrist and neuropathologist. Dr. Alzheimer performed a postmortem examination of the brain from a woman who had died of at that time/then unfamiliar mental illness. She had symptoms that included memory loss and language problems, accompanied with the unpredictable behavior. He described many

abnormal clumps (now called amyloid plaques) and tangled bundles of fibers (now called neurofibrillary, or tau, tangles) in the patient's brain. These unique pathological features in the brain are still considered some of the main hallmarks of Alzheimer's disease, although other features, such as the loss of connections between nerve cells (neurons) in the brain are considered as some of the AD pathological changes (Figure 2). This general malfunction of neuronal communication is responsible for the subsequent behavioral changes.

Although AD was discovered more than one hundred years ago, the full clarification of its pathogenesis is not yet achieved. Nevertheless, specific anatomical markers of AD are known and are helpful in understanding the disease pathogenesis and for the development of possible therapies. These are macroscopic damages such as the atrophy of specific brain regions, and microscopic changes, such as accumulation of irregular protein deposits (plaques), and the formation of specific structures - neurofibrillary tangles.

Although the brain atrophy most often starts in the hippocampus, a brain region responsible for the control of memory, it is now known that this process begins decades before the first symptoms. At the late stage of the disease there is a significant shrinkage of the brain. The formation of plaques and neurofibrillary tangles is the result of the abnormal production and processing of two brain proteins, Amyloid beta and Tau, that are the focus of the worldwide research efforts executed in the dozens of laboratories. Amyloid-beta (Aβ) is a fragment of a larger protein – amyloid precursor protein (APP). Irregular processing of this protein at the surfaces of neurons leads to the abnormal production of extracellular amyloid-beta fragments. When these fragments cluster together, they form larger deposits called amyloid plaques which appear to

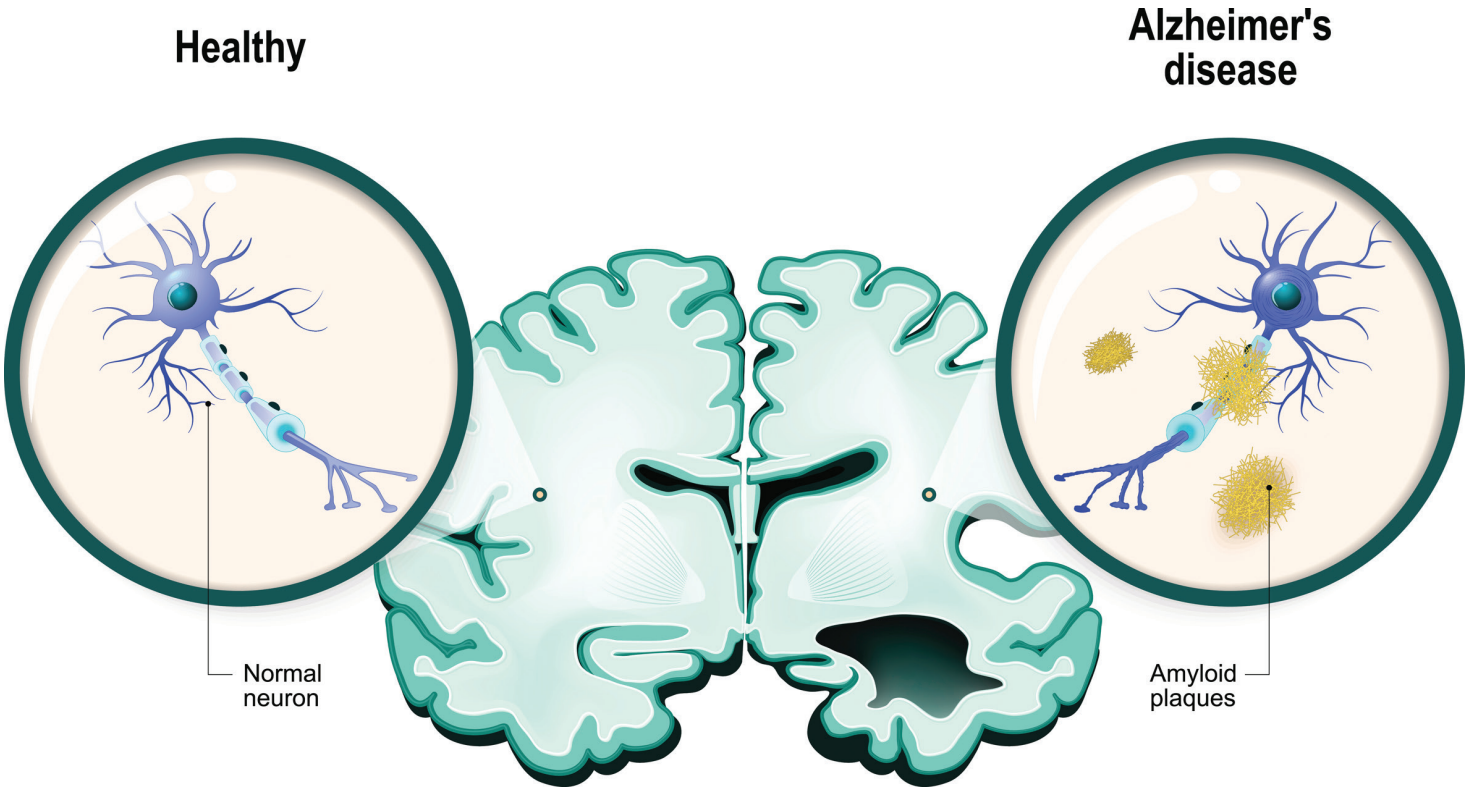


Figure 2. The brain of Alzheimer's patients is characterized with macro and micro changes. Macro changes include atrophy of specific brain regions and micro changes are the formation of amyloid plaques. (Image Credit: Designua/Shutterstock)

have a toxic effect on neurons and disrupt cell-to-cell communication. Tau proteins play a part in neuron's internal support and transport system. In Alzheimer's disease, due to hyper-phosphorylation tau proteins change shape and organize themselves into structures called neurofibrillary tangles. These tangles disrupt the transport system and are toxic to neuronal cells. How these changes occur and why they are present in some people and not in others remains still unsolved.

What causes Alzheimer's disease?

There are several different types of AD that are classified based on the time when symptoms start to appear - late onset (LOAD) and sporadic (SAD), or early onset (EOAD) and familial AD (FAD). FAD and EOAD is manly associated with genetic mutations in the amyloid beta precursor protein (APP), presenilin genes (PSEN1 and PSEN2), and apolipoprotein E gene (APOE). Although one variation of the APOE gene, APOE e4, increases the risk of Alzheimer's disease, and approximately 25% to 30% of the population carries this particular mutation, not everyone with this variation of the gene develops the disease. Nevertheless, although there are other genetic mutations that virtually guarantee that person who inherits them will develop AD, these mutations are responsible for less than 1% of people with AD. Thus, there are other factors that are necessary for AD to develop and they can be environmental, metabolic, viral etc. Nevertheless, aging remains the greatest risk for developing AD. Among health conditions, one of the significant risks for developing AD is mild cognitive impairment (MCI), characterized by a decline in thinking skills and memory that is greater than normal for a person's age, but MCI is not preventing normal social and work functioning. In addition, people who suffered a severe head trauma classified as *traumatic brain injury* (TBI) also have a greater risk for AD. Interestingly, this risk is the highest within the first six months to two years after the TBI suggesting a window of opportunity for the development of preventive therapeutic strategies.

Environmental factors can influence or cause AD as well, and the important one is air pollution. Studies in animals have indicated that air pollution can augment the degeneration of the nervous system. Human studies have also found that air pollution exposure - particularly from traffic exhaust and burning wood - is associated with a greater dementia risk. Researchers have also shown that consummation of large quantities of alcohol can be linked to the increased risk of dementia, particularly the early-onset dementia. Poor sleep patterns such as having difficulties falling or staying asleep are also associated with the higher risk of AD.

Finally, the large group of life style factors such as lack of exercise, obesity, smoking or exposure to secondhand smoke, high blood pressure, high cholesterol, and poorly controlled type 2 diabetes are also strongly associated with the increased risk of AD. The good news is that at least in case of these life style factors we have the power to alter the odds in our favor. For example, regular exercise and a healthy low-fat diet rich in fruits and vegetables are associated with a decreased risk of developing AD. Lastly, scientific studies have established a sound association between lifelong involvement in mentally and socially stimulating activities and a reduced risk of AD.

Diagnosis of Alzheimer's disease

The time from diagnosis to death varies depending on the age of the person. If the person is older than 80 when diagnosed the prognosis is 3 or 4 years, but if the person is younger than the prognosis can be as long as 10 or more years. Alzheimer's disease is currently ranked as the sixth leading cause of death in the United States. However, it can be ranked as third, according to the recent estimates, as a cause of death in elderly, just behind heart disease and cancer. The important part of diagnosing AD includes the ability of the patient to explain the symptoms, as well as the information obtained from family members, friends, and caregivers about symptoms and how are they affecting the everyday life. Finally, a diagnosis is based

on tests that doctor administers. These tests are designed to assess memory and thinking skills. The additional laboratory and imaging (PET scans) tests can also rule out other potential causes and/or help the doctor to identify more precisely particular symptoms important for diagnosing AD and to distinguish among similar symptoms that characterize other types of dementias such as vascular, senile and others.

Nonetheless, AD is only diagnosed with complete certainty after death when the postmortem microscopic examination confirms the presence of specific plaques and tangles in the patient's brain.

Treatment of Alzheimer's disease

Although treatment can help manage AD symptoms in some people, currently there is no cure for this devastating disease.

Current medications prescribed for the treatment of AD can initially help with memory symptoms and other cognitive changes. There are two types of drugs in use for the treatment of AD - cholinesterase inhibitors and memantine (Namenda). Cholinesterase inhibitors work by increasing levels of cell communication through the preservation of a specific messenger molecule (acetyl choline) that is depleted in the AD brain. Most people see modest improvements with this treatment. However, these drugs have serious side-effects such as diarrhea, nausea, loss of appetite and sleep disturbances, and in some cases cardiac arrhythmia. Memantine (a NMDA receptor antagonist) works on another line of communication in the brain and this treatment is able to slow the progression of symptoms in moderate to severe cases of AD. Relatively rare side effects include dizziness and confusion. Sometimes these two treatments can be combined.

Therefore, the choice of treatments that AD patients face after diagnosis is very limited and the outcome is not very prospective - they decelerate, but not cure the disease. Because of that, the development of new treatments has been an urgent focus of research labs and clinics in the last decades. A number of governmentally and privately funded grants exist with a sole mission of finding the cure for AD. They invest in all the studies that carry the hope of finding a solution for this debilitating disease that is more than a century present in human population.

Nano-technology applications – Fullerenes

Nowadays, the nano-technology is omnipresent in our everyday life. Therefore, with the urge of discovering new treatments for AD, it does not come as a surprise that attempts are made to harness the potential of this powerful methodology if not to cure, than to diminish/curtail/contract the devastating effects of this disease. One such study examined the effects of a patented fullerene derivative, hyper-harmonized-hydroxylated fullerene water complex - 3HFWC (developed in TFT Nano Center, Belgrade as a part of ZEPTER GROUP International) (Figure 3). Fullerene is the third pure crystal carbon form, besides graphite and diamond. It is an allotrope (a structurally different form of the same element) of carbon whose molecule consists of carbon atoms connected by single and double bonds forming a closed or partially closed mesh, that consist of fused rings that contain five to seven atoms. The molecule may be a hollow sphere, ellipsoid, tube, or many other shapes and sizes. The fullerene family is named after buckminsterfullerene (C_{60}), the most famous member, which in turn is named as an homage to Buckminster Fuller, an American architect, systems theorist, author, designer, inventor, and futurist (who used the similar structural principles for architectural objects that he designed). The closed fullerenes, especially C_{60}, are also informally called buckyballs for their resemblance to the standard soccer ball.

Fullerenes had been predicted for some time, but only after their accidental laboratory synthesis in 1985 were they detected in the nature and outer space. Sir Harold Kroto, Robert Curl and Richard Smiley were awarded the 1996 Nobel Prize in Chemistry for the discovery of this class of molecules.

Since then fullerenes have been the subject of intense research, both for their chemistry and for their technological applications, especially in materials science, electronics, and nanotechnology.

C_{60} is able to produce a large number of derivatives. Considering that fullerenes have pour water solubility, the focus was put on developing chemical modifications such as the addition of hydroxyl groups creating fullerenols, compounds soluble in water.

Therapeutic properties of fullerene and its derivatives were first shown in 1993, when Friedman and colleagues, demonstrated their ability to inhibit the activity of HIV protease, the key enzyme responsible for the formation of infectious HIV virus. The other important property of these compounds is the ability to inhibit bacterial binding to the host cells through the interaction with the adhesive molecules on the membrane. These compounds can interact with the immune system and cancer cells. It was shown that they can inhibit the growth of specific cancers in experimental conditions but to also modulate the activity of some cytotoxic drugs. One of the most important characteristics of fullerenes is that they have anti-oxidative properties absorbing free radicals and protecting cells from their toxic effects.

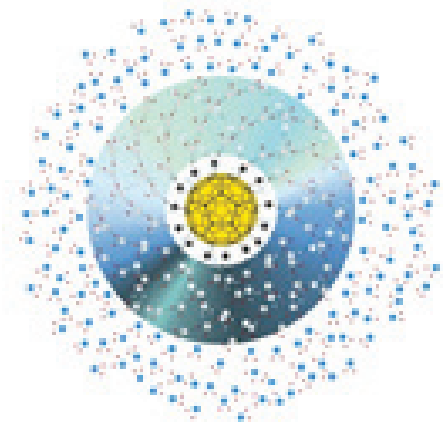


Figure 3. 3HFWC, Hyper harmonized hydoxylated fullerene water complex $C_{60}(OH)_x$ @ $[(H2O)^l]^{\Phi/\phi}$

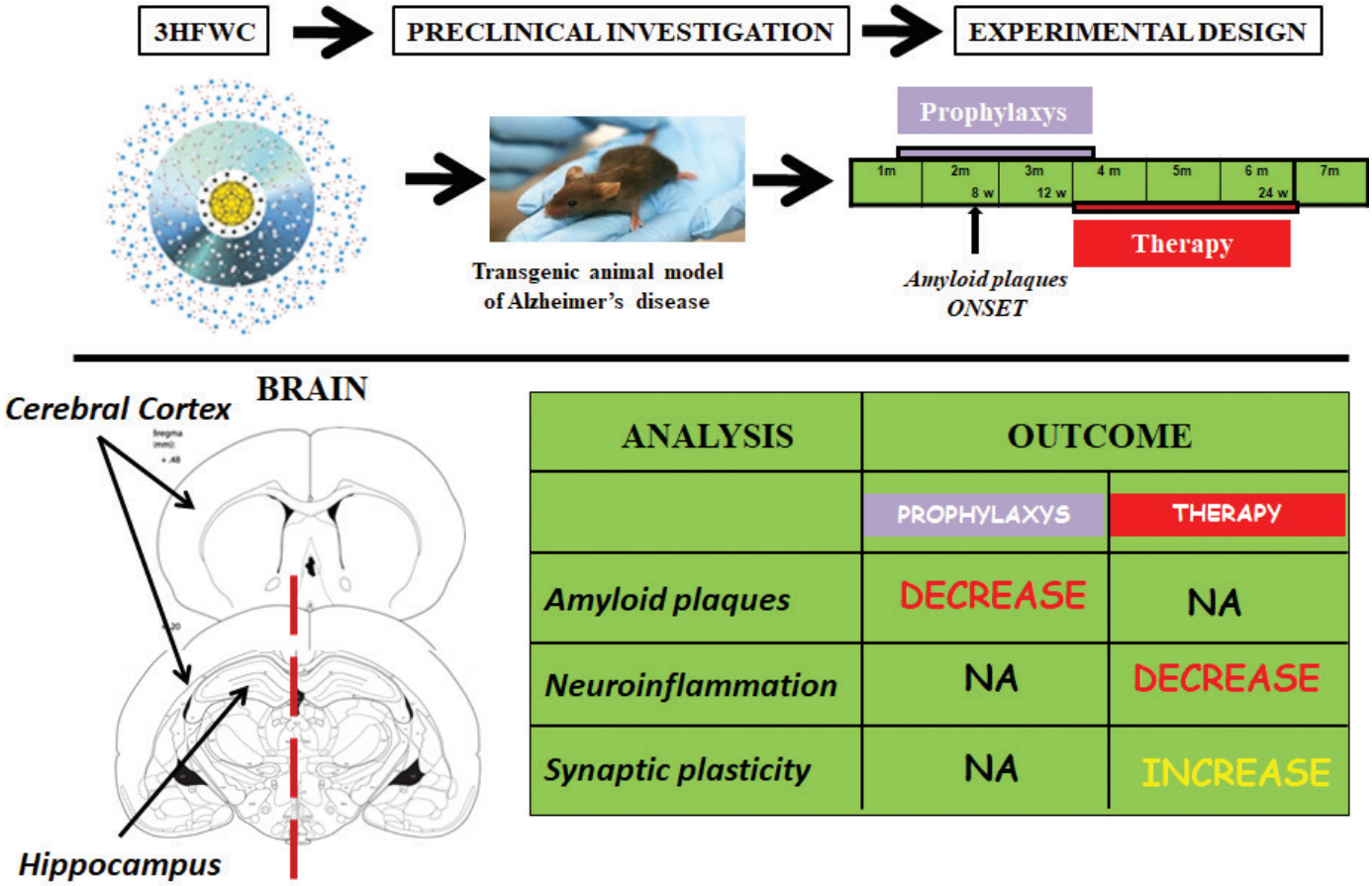
However, fullerenes can be activated with different wavelengths of light generating free radicals and damaging membranous lipids and proteins. This ability of fullerenes and its derivatives is used as the base for photo-dynamic therapy where fullerenes act as sensitizing substances and exert beneficial effects in different systems.

In order to understand the ability of 3HFWC to interact with amyloid-beta (Aβ), the culprit protein of AD, numerous studies were done, mostly in solution and in the cell culture conditions (i.e. *in vitro*). These studies showed that water-soluble fullerenes are able to interact with Aβ protein's 3D structure inhibiting their aggregation, the same process that initiates the formation of plaques.

Fullerenes' potential in AD: the results from animal studies

The potentially beneficial actions of 3HFWC fullerene on the development and progression of AD was analyzed in the brains of experimental animals, rodents, in in vivo conditions. Considering that rodents do not develop AD, scientists have designed specific genetic modifications in mice in order to mimic the development and progression of familial Alzheimer's disease (FAD). One such model is 5xFAD mouse that bears 5 mutations in 2 genes whose altered functions are implicated in the development of AD. These genes are amyloid precursor protein (APP) and presenilin 1 (PSEN1). Previous studies have established that 3HFWC given orally, dissolved in drinking water, did not have any overall harmful effects on mice. However, fMRI scans of the CNS in treated wild-type mice showed increased activity of specific brain regions when compared to the control mice. The next step was to determine the effects of 3HFWC in an animal model of AD, the 5xFAD transgenic mice. The important fact to take into account when looking for the potential treatment for AD is that in humans, this disease can start many years before visible symptoms.

This period when the disease has already started but the patient is asymptomatic is



ANALYSIS	OUTCOME	
	PROPHYLAXYS	THERAPY
Amyloid plaques	DECREASE	NA
Neuroinflammation	NA	DECREASE
Synaptic plasticity	NA	INCREASE

Figure 4. The effect of 3HFWC nano-substance, administered as a prophylaxis or therapy, on amyloid plaque burden, neuroinflammation and synaptic plasticity in 5xFAD transgenic animal AD model.

called *prodromal* phase. Mice have shorter life span and in AD transgenic models the prodromal phase lasts several months, facilitating the evaluation of putative new therapies. With that in mind, the specific study was designed aiming to evaluate and compare the effects of 3HFWC when it is administered in prodromal phase (before the appearance of amyloid plaques), or when animals were already sick. Both groups were treated for 3 months. Interestingly, this study showed that although 3HFWC had beneficial effects on specific aspects of AD pathology in both experimental paradigms, these effects strongly depend on the time when the treatment was given (before the symptoms of a disease were visible or when the disease is fully developed). When approaching such a complex disease as AD, several aspects of brain pathology are taken into account and the effects of the new treatment are evaluated on each of them.

Firstly, the main structural parameter analyzed is the formation of amyloid plaques, an irregular accumulation of missfolded protein aggregates that is the hallmark of AD. A decrease in plaque accumulation or in the presence of its main constituent, the toxic form of amyloid-beta peptide ($A\beta_{42}$) is considered beneficial for the treatment of AD. Secondly, the pathological changes in AD brain such as the formation of plaques and tangles are accompanied with the increased inflammation that can induce further damage to the nervous tissue. Therefore, diminishing the neuroinflammation is always beneficial. Lastly, the proper nerve cells communication is necessary in order to successfully respond to everyday tasks, to solve problems, and to think. This communication is achievable through specific structures called synapses. They are adaptable, dynamic and responsive structures representing the foundation of brain's plasticity, a

unique feature responsible for our adaptive responses to various intrinsic or extrinsic stimuli. The dynamic nature of synapses, i.e. their plasticity, can be measured and used to evaluate the level of pathology or the efficacy of the potential therapy. Importantly, synaptic plasticity is weakened in neurodegenerative disorders, including AD and the treatment is considered beneficial if it induces the increase in synaptic plasticity. The effects of 3HFWC were analyzed in two brain regions that are regularly affected in AD, the cortex and hippocampus, and are responsible for thinking and for the formation of memories.

The effects of 3HFWC in the brain of an animal model of Alzheimer's disease

The analyses of experimental animals (40 mice) after the 3-month-long 3HFWC treatment showed clear and significant differences when compared to control transgenic animals and importantly, showed qualitatively different effects whether 3HFWC was given as a *prophylaxis* or as a therapy. When 3HFWC was given as prophylaxis, i.e. during the prodromal phase, to the animals that are in the early stages of the disease, the number of plaques in the brain was reduced while the neuroinflammation and synaptic plasticity were unaltered. In contrast, when 3HFWC was given as a *therapy* to the already sick animals the effect on amyloid plaques was missing, but neuroinflammation was significantly reduced and synaptic plasticity increased (Figure 4).

The prophylactic effect of 3HFWC on the level of plaque formation is most probably the result of the conformational changes of amyloid-beta affecting its irregular aggregation. The anti-inflammatory effect of the treatment with 3HFWC when the disease is already developed in full and when the plaque growth is in its exponential phase may act as an indirect support of the synaptic health, increase its plasticity, and as a result improving the cognitive functions in general. In this case, the possibility that 3HFWC can be used as an adjuvant to conventional therapies in order to improve patient's mental health or to slow down the progression of disease is exciting. As in the case of prophylactic use the therapeutic potential of 3HFWC warrants more in-depth studies.

Conclusion

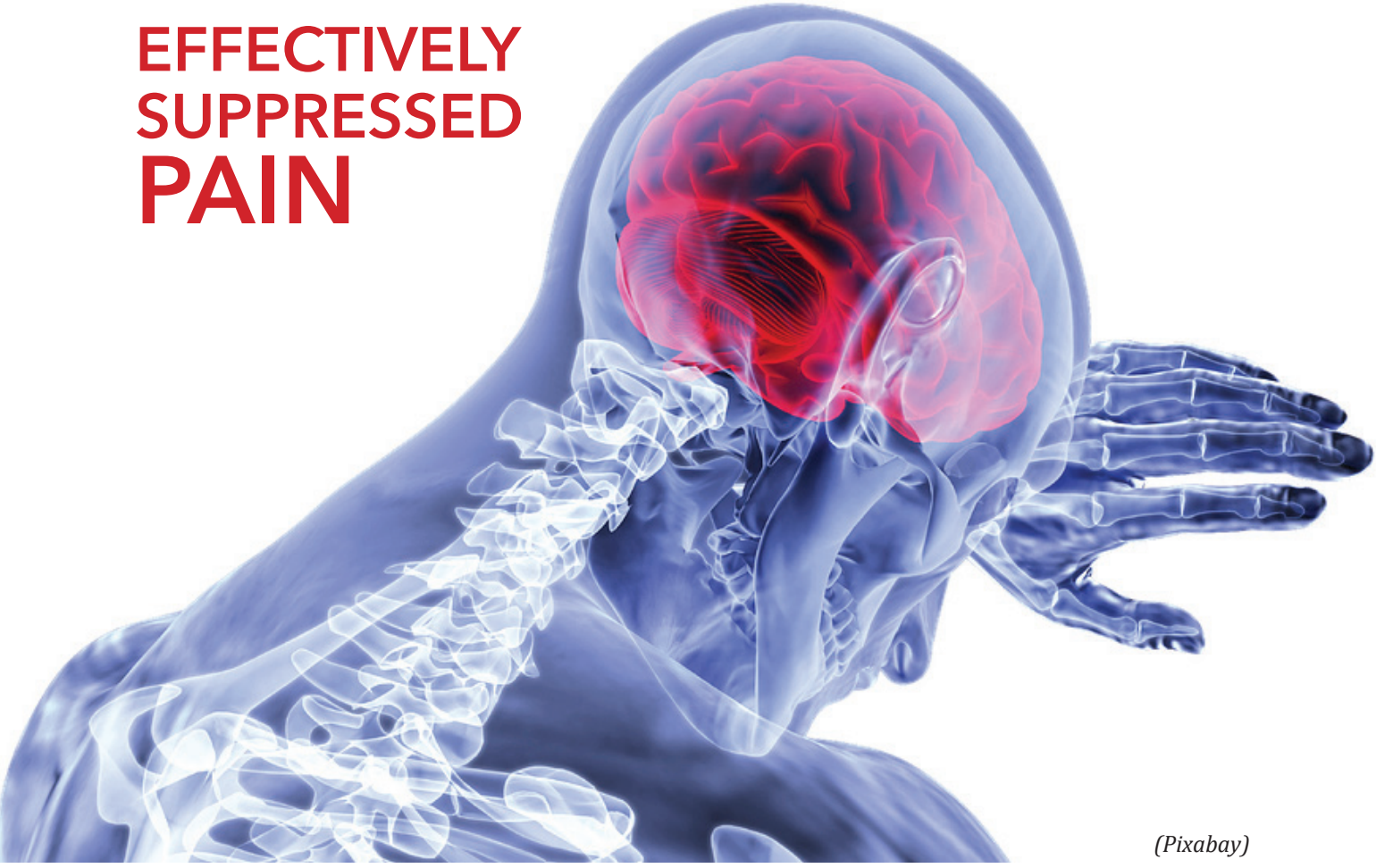
AD belongs to the class of diseases that have extracellular protein aggregates as a common denominator. Specific proteins become structurally abnormal and consequently impair the function of cells, tissues and organs in the body. Such irregular proteins can become toxic or they can lose their normal function. In medicine, these diseases are called *proteinopathies* and they include besides AD, such diseases as Creutzfeldt-Jakob disease and other prion diseases, Parkinson disease, amyloidosis, multiple system atrophy and a wide range of other disorders. The development of effective treatments for many proteinopathies has been difficult as they not only involve different proteins but they arise often from different sources.

The potential of fullerenes to interact with Aβ and to exert an effect on the diseased AD brain in experimental animals is particularly important considering that extracellular irregular protein aggregates are also present in the specific age-related form of neurodegeneration defined as mild cognitive impairment (MCI). The potential of 3HFWC to slow down protein aggregation and decrease the speed of subsequent neurodegeneration is intriguing. These findings substantially broaden the potential therapeutic value of 3HFWC, particularly as a general prophylactic in aging population. However, further studies are necessary to fully understand the effects of this nano particle in ameliorating the consequences of aging. Some of the exciting questions that need answers are - what will be the effect of fullerenes on other proteinopathies in experimental animals; what, if any, are the effects of fullerenes on other organs not primarily affected with the specific disease; can fullerenes be considered as potential adjuvants for the already existing therapies?

EFFECTIVELY SUPPRESSED PAIN

White (polychromatic) light and its individual color (monochromatic) components in the wavelength range from 400 to 780 nm have been received by humans for medical purposes for many centuries. This physiotherapy direction has the general name actinotherapy with variations of light therapy, phototherapy, low-intensity laser therapy, color therapy, photobiomodulation.

There are no objections to the empirically established approaches to the treatment of psychoemotional disorders, insomnia, neonatal jaundice, prophylactic and pain-relieving light procedures, etc. [1-6]. The possibilities of light therapy have expanded since the advent of LASERs, which has drawn particular attention to a new kind of light. Laser creates coherent polarized light (monochromatic), the quanta of which propagated in a parallel stream. High-intensity laser light is used for the surgical destruction of pathological foci [7]. Low-intensity laser radiation is successfully used for therapeutic purposes in der-



(Pixabay)

We revealed that the factors that significantly enhance the analgesic result of PL therapy are the polarization of light and the presence of the long-wavelength part of visible light. Clinical observations of human pain before and after the application of PL revealed dynamics similar to those obtained in animals. Analgesia after 3-day application of red light to AP He-Gu in a patient with post-traumatic pain reached 48.2%. We emphasize the efficacy, simplicity and safety of the PL color clinical application.

Sergiy A. Gulyar, Zynaida A. Tamarova