ALZHEIMER'S DISEASE: PATHOPHYSIOLOGY AND TOWARDS RATIONAL DRUG TREATMENT.

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Abstract

During the past two decades, one of the foremost challenges in health research was to understand better the cause(s) of AD for the development of safe and effective pharmacological treatments. However, irrespective of the form of therapy, the current approaches for the treatment of AD provide only temporary symptomatic relief, improve cognitive function, but do not slow the long-term progression of this disorder with several side effects. Moreover, these treatments have a modest effect on the progression of AD from Mild cognitive impairment, (***MCI***), to disabling dementia and death. Therefore, there is an urgent need to develop strategies to improve the efficacy, the transport across the blood brain barrier (BBB), the bioavailability, and subsequently to limit the adverse effects of pharmaceutical compounds for the treatment of AD. Nanotechnology represents an expanding strategy in this regard and promises advances to the diagnosis and may provide a possible solution to overcome challenges for the treatment of AD. An overview of the state of the art of development of AD pharmacotherapy and novel nanomaterials that have potential to improve diagnosis and therapy of AD and have been tested in different experimental models for delivery of drugs will be discussed.

Abbreviations:

MCI-Mild cognitive Impairment, AD-Alzheimer's Disease, AB-Amyloid beta,

NT-Neurofibrillary tangles.

1.1 Alzheimer's Disease Statistics

A global epidemic of Alzheimer's disease (AD) is occurring as the world's population ages[1]. The prevalence of AD increases from ~3% at the age of 65 years to ~47% at the age of 85 years. In 2010, there are 3.7 million Indians with dementia [2]. The worldwide prevalence of AD was 26.6 million, and by 2050 the prevalence will quadruple. It is the leading cause of persistent dementia in late life. Survival for a decade is common.

1.2 Stages of AD

i) Predementia: Subtle problems with the executive functions of attentiveness, planning, flexibility, and abstract thinking, or impairments in semantic memory (memory of meanings, and concept relationships) and Mild cognitive impairment.

ii) Early AD: There is increasing impairment of learning and memory. Language problems (shrinking vocabulary) are noticed .Difficulty in memorizing new facts or happening

iii) Moderate AD: Unable to perform most common activities of daily living. Behavioral and neuropsychiatric changes become more prevalent. Impaired short term as well as long term memory.

iv) Advanced AD: Patient is completely dependent upon caregivers. Complete loss of speech.

Diagnosis is usually confirmed by assessments of behavior and cognition. As the disease progresses, confusion, irritability, aggression, mood swings and withdrawal become commonplace. The disease excludes individuals from maintaining normal life events and in the latter stages of disease often requires long-term care and institutionalization[3].

Histological examination is the only way to obtain a definite diagnosis for this pathology[4].

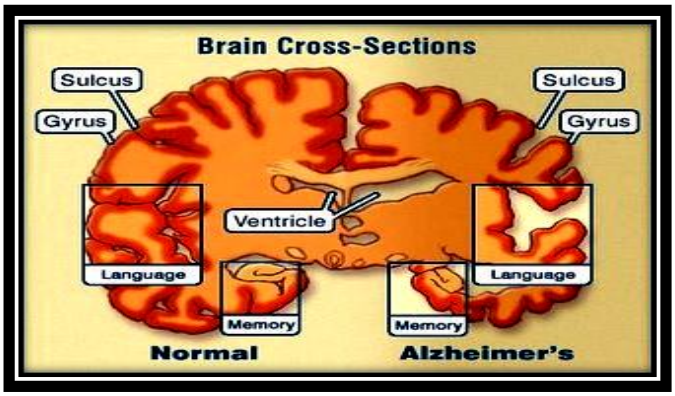

Figure 1

2. PATHOPHYSIOLOGY OF AD:

Pathologically, AD is characterized by loss of cortical, and to a lesser extent, sub cortical neurons and synapses(fig.2).This results in gross atrophy, including degeneration of the temporal and parietal

lobes and parts of the frontal cortex and cingulate gyrus.

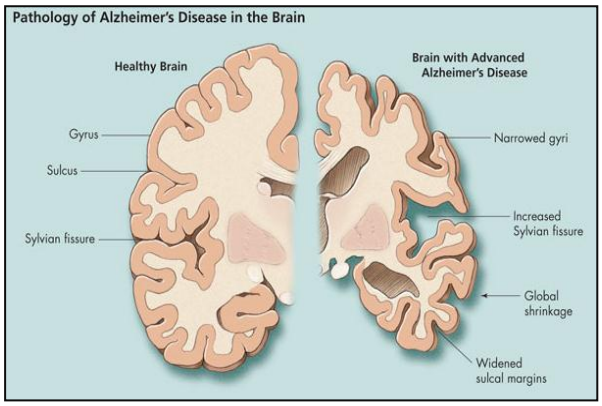

Figure 2: Pathology of Alzheimer's disease in the brain

The two types of characteristic lesions are Extracellular senile plaques (**SP**) and Intracellular neurofibrillary tangles **(NT)** (fig. 3)

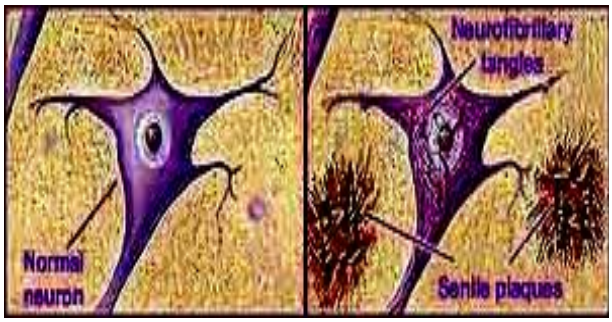

Figure 3: Characteristic Lesions in AD

2.1 Extracellular senile plaques (SP)

SP are composed of abnormal aggregations of amyloid-β (AB) [1]. Amyloid plaques are relatively insoluble dense cores of 5-10 nm thick amyloid fibrils with a surrounding "halo" of dystrophic neurites, reactive astrocytes and activated microglia. The main proteinaceous component of amyloid plaques is the AB peptide. AB is not a single molecular entity, but rather is composed of a family of peptides produced by proteolytic cleavage of the type I transmembrane spanning glycoprotein AB precursor protein (APP).Once released by proteolytic cleavage, the AB peptide may exist in solution and can be detected in CSF and plasma. Extensive investigations using behavioral models, neuronal cultures and APP knockout mice suggest that APP may serve as a receptor for and appears to play a role during axonal regeneration and as a regulator of neural activity, connectivity, plasticity and memory[3].In addition, it promotes the adhesion of cells to their substrate (Schubert et al., 1989) and protects neurons against excitotoxic and oxidative injury[5].

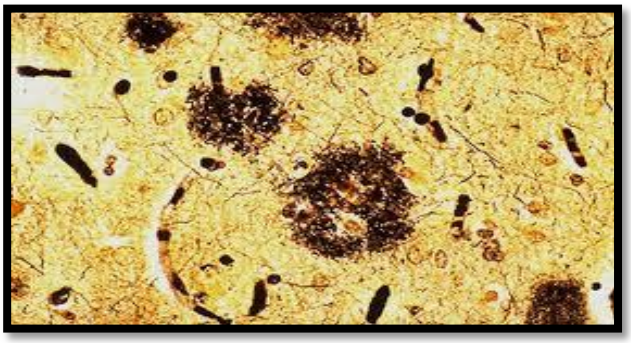

Figure 4

2.1.1 The Amyloid Beta Protein (AB)

The major protein component found in amyloid deposits is a 4 kDa AB protein .The AB peptide is a proteolytic product of the much larger ~100–130 kDa protein, called the amyloid precursor protein (APP). Complete sequencing of the AB peptide has shown that it consists of 39–43 amino acid residues. Before cleavage from the APP molecule, the N-terminal 28 residues of AB are extracellular and the remaining residues are located within the transmembrane domain. C-terminal 12 amino acid residues of the AB peptide are hydrophobic (Fig. 5) and endow the peptide with the ability to self-aggregate and polymerize into amyloid fibrils. Beside AD, there are several other human diseases with amyloidosis, such as type II diabetes mellitus[6].

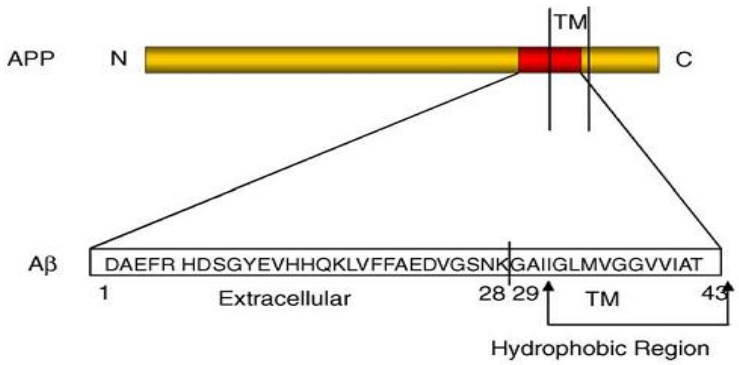

Figure 5: Structure of AB Protein

2.1.2 Trafficking and Proteolytic Processing of Amyloid Precursor Protein

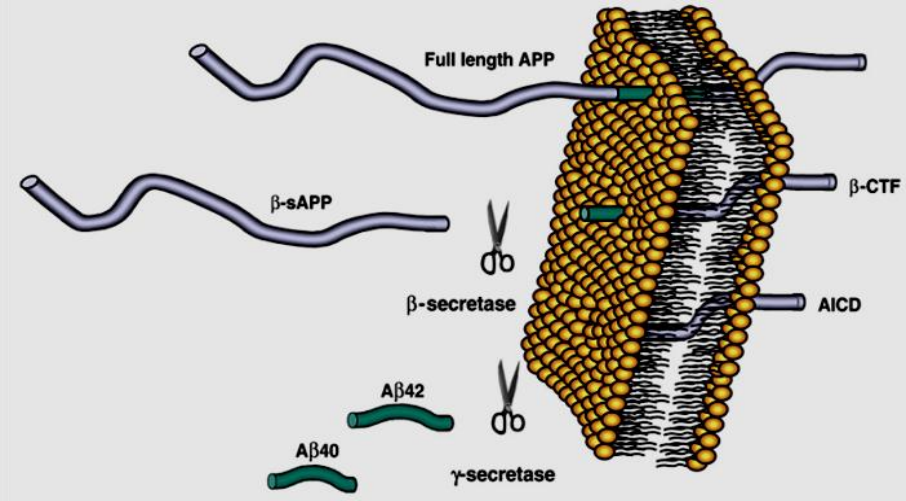

Figure 6: APP Proteolytic Processing

The majority of mature APP is proteolytically cleaved via one of two competing pathways, the non-amyloidogenic and amyloidogenic pathways. The APP is an integral membrane protein processed by the three proteases-alpha, beta, and gamma secretase, which have been implicated in the cause of AD. **Beta-Secretase** generates the -NH_2 terminus of AB, cleaving APP to produce a soluble version of APP (Beta-APPs) and a 99-residue –COOH terminal fragment (CT99) that remains membrane-bound[6]. In contrast, **Alpha-secretase,** which cleaves on the C-terminal side of residue 16 of the AB sequence to produce APP alpha-s, an 83-residue COOH-terminal fragment (CT83). Both CT99 and CT83 are substrates for **Gamma-secretase**, which performs an unusual proteolysis in the middle of the transmembrane domain to produce the 4-kDa Beta-Amyloid (AB) and CT57–59 [amyloid intracellular domain (AICD)] from CT99, and a 3-kDa peptide called p3 and CT57–59 from CT83. The processing of the APP C-terminal fragment by gamma-secretase is mediated by the presenilins. Proteolysis by Gamma-secretase is heterogeneous, a 40-residue peptide (AB_{40}), whereas a small proportion is a 42-residue COOH-terminal variant (AB_{42}). The longer and more hydrophobic AB_{42} is much more prone to fibril formation than is AB_{40} and even though

AB_{42} is a minor form of AB, it is the major AB species found in cerebral plaques[7].

2.2 Neurofibrillatory Tangles (NT)

A second defining pathological hallmark of AD is the formation of (NTs), which are insoluble filamentous accumulations found in degenerating neurons. They are composed of a cytoskeletal protein called 'tau', which normally binds to microtubules and regulates their state of polymerization. In AD, tau becomes hyper phosphorylated and self-aggregates, leading to microtubule depolymerisation and degeneration of dendrites and axons in neurons. NTs are frequently localised in the hippocampus, entorhinal cortex, amygdala and the perirhinal cortex[6].

2.3 Genetic Risks of AD

Although most AD cases belong to the group of so-called late onset or sporadic forms of AD, there is also a genetic component of this disorder. Interestingly, a familial accumulation of AD has been reported as early as 1934 .Today, it is well-known that 10% of AD patients have some family history. A total of four genes have, so far, been implicated in the pathogenesis of AD: the genes for the AB precursor protein (APP), for presenilin 1 and 2 (PS1, PS2), and the gene for apolipoprotein E (ApoE). The ApoE4 allele is associated with both familial and sporadic late-onset AD.

2.4 Metals Imbalance and Biochemical Changes in AD

In **Normal Physiological Condition**, the vesicular zinc transporter ZnT3 transfers zinc into synaptic vesicles. Upon stimulated release zinc concentrations can achieve 300 mM within the synaptic cleft. Copper is released post-synaptically following N-methyl-D-aspartate (NMDA)-induced activation, which causes the translocation of ATP7a and its associated copper-laden vesicles to the synaptic cleft. Both copper and zinc are able to inhibit the NMDA receptor response, which may feedback to prevent further copper from being released into the cleft. AB would typically be cleared by movement into the periphery or degradation by extracellular proteases such as neprilysin and insulin degrading enzyme (IDE). Despite high peak concentrations upon neuronal stimulation, the average concentrations of free synaptic copper and zinc is kept low over time by a variety of other ways including putative energy-dependent reuptake mechanisms as well as buffering

by Metallothioneins (e.g. MT3) from neighboring astrocytes.

In **Alzheimer's disease** there is decreased mitochondrial energy which leads to reduced metal reuptake, which causes the average concentration of metals to rise over time. This allows copper and zinc to react with AB released into the synaptic cleft to form oxidized, cross-linked soluble oligomers and precipitated amyloid. AB can bind up to 2.5 moles of metal ions, but becomes more densely aggregated as it becomes loaded with zinc. While the soluble AB monomers are constitutively degraded, zinc-loaded AB oligomers are resistant to degradation. MT3 is also decreased in AD, so promoting abnormal metal-AB interaction and sequestered metal ions by AB allows unopposed glutamate activation of the NMDA receptor, which could lead to the increased release of post-synaptic copper[8].

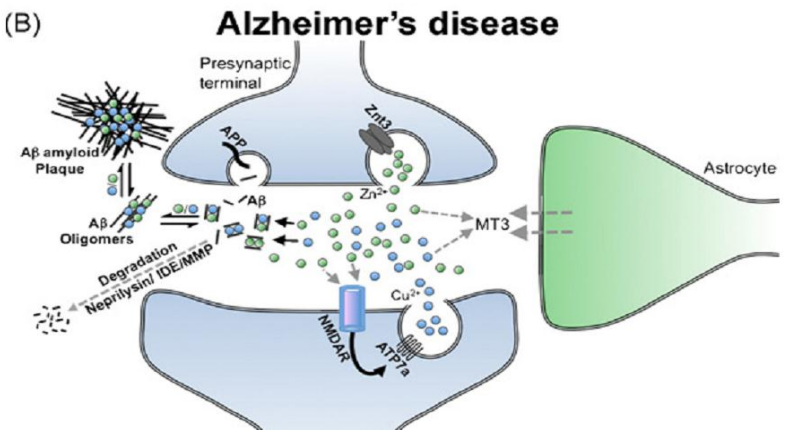

Figure 7: Metal Imbalance in AD

3. Challenges in Drug Delivery for AD:

The targeted drug delivery to the central nervous system (CNS), for the diagnosis and treatment of neurodegenerative disorders such as AD, is restricted due to the limitations posed by the blood brain barrier (BBB) as well as due to opsonization by plasma proteins in the systemic circulation and peripheral side-effects[3].

3.1.1 Transport mechanisms of the BBB

a) Paracellular diffusion

The tight junctions between endothelial cells results in a very high transendothelial electrical resistance of 1500-2000 Ω cm^2 compared to 3-33Ω cm^2 of other tissues

which reduces the aqueous based paracellular diffusion that is observed in other organs.

b) Transcellular diffusion in brain capillaries, intercellular cleft, pinocytosis, and fenestrae are virtually nonexistent;

exchange is mainly transcellularly. Therefore, only lipid-soluble solutes that can freely diffuse through the capillary endothelial membrane may passively cross the BBB.

c) Absorptive-Mediated Endocytosis

Absorptive-mediated transrytosis is triggered by electrostatic interactions between the positively charged moiety of the peptide and the negatively charged plasma membrane surface region.

d) Receptor-Mediated Endocytosis

To get molecules to cross the BBB they need to be manipulated in a manner such that penetration into the brain is achieved. Sometimes this is achieved by synthesis of chimeric peptides. They are formed by covalent binding of the non-permeable but pharmacologically effective portion of the peptide to an appropriate vector that can be transported across the BBB. The intact chimeric peptide is transferred into the brain's interstitial space by receptor-mediated exocytosis. Subsequently, the binding between the vector and the pharmacologically active peptide is cleaved and, finally, the released peptide exerts its pharmacological effect in the brain. It occurs at the brain for macromolecular substances, such as transferrin, insulin, leptin, and IGF-I&IGF-II, and is a highly specific type of energy dependent transport[9].

e) Carrier mediated transporter (CMT)

Carrier mediated transporter (CMT) system is expressed on both the luminal and abluminal membranes of the brain capillary endothelium and operates in both directions, i.e., from blood to brain and brain to blood directions .The CMT systems can be exploited for brain drug-delivery after reformulating the drug in such a way that the drug assumes a molecular structure mimicking that of the endogenous ligand (glucose, amino acids). For example, pseudonutrients are the polar small drug molecules which are made by mimicking the structure of nutrients[9].

4. THERAPEUTIC APPROACHES:

Alzheimer's disease is highly prevalent and well characterized, with a number of potential therapeutic options but regrettably few currently in clinical

practice[3]. AD therapy would improve substantively if drugs could be delivered specifically to affected brain areas. Therapy could also improve diagnostics if plaques, tangles and/or neuropathological activities could be seen earlier in the disease course. These include, but are not limited to Nineteen compounds are currently in Phase II trials, out of which three compounds (AN1792,lecozotan SR, and SGS742) failed at this stage of development. There are many more candidate molecules that are at the pre-clinical stage of development and are likely to proceed into clinical trials based on the cholinomimetic therapy, the amyloid cascade, the metal and the oxidative stress mechanisms[3].

4.1 Acetylcholinesterase Inhibitors

Profound losses in the cholinergic system of brain, including dramatic loss of choline uptake and ACh level in the neocortex and hippocampus and reduced number of the cholinergic neurons in basal forebrain and nucleus basalis of Meynert, are closely associated with cognitive deficits observed in the disease. Blocking acetylcholine hydrolysis with AChEI is the most popular approach. After the FDA approved **Tacrine** in 1993, several kinds of AChEIs such as **donepezil, galantamine, and rivastigmine** have become available for the symptomatic treatment of patient with mild-to-moderate AD. However, weakness of such AChEIs caused by limitations related to short biological half-life, transient and weak effects, narrow therapeutic range, low BBB, and frequent adverse effects block their way to treating cognitive deficits in AD. Cholinergic-based therapy using AChEI is currently known to be the best clinical approach for improving cognitive deficits in AD[7].

Table 1 gives details of various AChE inhibitors

Feature	Tacrine (Cognex)	Donepezil (Aricept)	Galantamine (Reminyl)	Rivastigmine (Exelon)
Dose	40mg/day	5mg/day	8mg/day	6-12 mg/day
Dosage Frequency	4 times daily	Once daily	Twice daily	Twice daily

Side Effects	Severe Hepatotoxicity, Nausea, Vomiting.	Anorexia, Nausea, Vomiting at Higher doses.	Nausea, diarrhoea, Abdominal Cramps at higher doses	GI upset, Weight loss, Vomitting, Dyspepsia

Table 1

4.2 Glutamatergic-system Modifiers

Overstimulation of the N-methyl-D-aspartate (NMDA) receptor by glutamate leads to neuronal calcium over-load and is implicated in the neuronal death characteristic of AD. Conversely, physiologic activation of the NMDA receptor appears to be necessary for normal cognitive function. **Memantine,** a noncompetitive (channel-blocking) antagonist with moderate affinity for the NMDA receptor, appears to block pathologic neural toxicity associated with prolonged glutamate release without blocking physiologic activation of the

NMDA receptor. It is approved by FDA in Severe Alzheimers[10].

4.3 Therapeutics and diagnostic targeting of metal ions in AD

One logical and increasingly popular theory for the use of neurotherapeutic small molecules in AD is to target the initiating event in the generation of free radicals[8]. As a preventive approach antioxidant molecules may be used for their ability to neutralize free or incorrectly bound metals, thereby interfering with the 'down-stream' generation of reactive oxygen species and other radicals. Numerous molecules with antioxidant properties, such as estrogen, melatonin, vitamin C and E (L-ascorbate and -topopherol, respectively), ginkgo biloba extract, curcumin and flavonoids have neuroprotective effects against AB-induced toxicity in cell- based experiments and animal models [3, 4].

4.4 AB Vaccine therapy

Immunotherapy targeting the AB peptide is a leading approach to disease-modifying treatment. AB antibodies might bind and remove small AB aggregates in the brain, thereby neutralizing the effects of toxic AB species on synapses. In an alternative mechanism, strategy is based on the binding of AB peptide in the blood that would "draw" the peptide from the brain through the BBB, possibly by a receptor-mediated process thus resulting in an increase in AB efflux from the brain to the periphery. The **heparin, gelsolin, and other molecules** are thought to "sink" or trap AB peptide in the blood and, at least

in animal model, reduce AB accumulation in the brain. As **amyloid vaccine AN-1792** led to the development of **Aseptic meningoencephalitis** in 6% of the patients, a second-generation vaccine, ACC-001, with an improved safety profile (with a short AB sequence as the immunogen, presumably preventing the induction of a toxic cellular immune response), was shown to be safe in a Phase I study and is currently in Phase II clinical trials with 360 patients with mild to moderate AD[4].

4.4.1 Passive AB immunization

The furthest along in clinical testing is **Bapineuzumab (AAB-001),** a humanized monoclonal antibody that recognizes the amino terminus of AB; with the Phase II a study yielding some encouraging results, particularly in the subgroup of patients not carrying the ApoE4 allele and the Phase III trial is under investigation.

4.5 Anti-inflammatory Therapy

Major support for the theory that inflammation contributes to neurodegeneration and that suppression of inflammation may therefore be beneficial comes from epidemiological studies. COX-1 is expressed in brain microglia and may be upregulated in some regions of AD brain, it may be necessary for effective suppression of antiinflammatory activity in the AD brain. In experimental models, neurotoxic stress, including ischemia and excitotoxicity and apoptosis is associated with upregulation of neuronal COX-2[11].

4.5.1 Curcumin

Curcumin (diferuloylmethane) is a low-molecular-weight, natural polyphenolic compound that is isolated from the rhizome of turmeric (*Curcuma longa*). It has a low intrinsic toxicity but a wide range of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, antiamyloid, and antitumor properties. The yellow curry spice is part and parcel of Indian vegetables[4].The incidence of AD in India is remarkably low compared to the U.S; this intriguing finding could be related to widespread use of curcumin in India[11].

4.6 Nanotechnology Based Therapeutic Approaches

A paradigm in cerebral drug targeting is by using particulate carriers. Nanoparticles are advantageous as they possess the high drug-loading capacities, thereby increasing intracellular delivery of the drug; the solid matrix of particulate carriers protects the incorporated drugs against degradation,

thus increasing the chances of the drug reaching the brain and Carriers can target delivery of drugs, and this targeted delivery can be controlled. One additional benefit of nanocarriers is that their surface properties can be manipulated in such a way as to evade recognition by the macrophages of the reticuloendothelial system (RES), hence improving the likelihood of nanoparticles reaching the brain[12].

4.7 Cholinesterase inhibitors-loaded NPs Polymeric NPs

4.7.1 Tacrine-loaded Chitosan NPs

Chitosan is a natural polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine, used as a safe excepient in drug formulations for over two decades; biocompatibility and biodegradability, make it a very attractive substance for diverse applications in pharmaceutical field. Chitosan has a positive charge when compared with many other natural polymers and is mucoadhesive. Wilson et al. prepared Tacrine-loaded chitosan NPs by spontaneous emulsification process. The biodistribution studies of drug-loaded NPs (drug to polymer ratio 1:1) were then carried out in rats. Tacrine freely crosses the BBB; a delivery system that releases the drug in a sustained manner as well as prolongs the residence time in blood may be helpful to improve the bioavailability of drug in the brain[4].

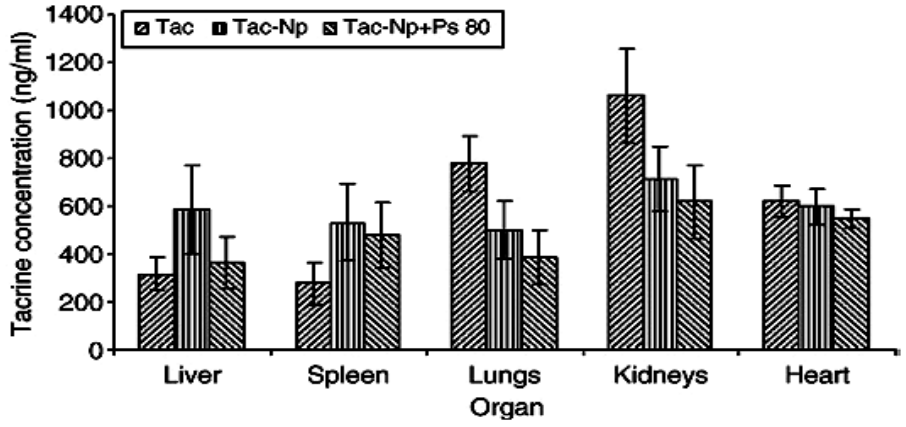

Figure 8: Tacrine concentration(ng/ml) in different organs after Tacrine-loaded chitosan NPs administration.

4.7.1.1 Tacrine loaded (PBCA) NPs

Targeting tacrine in the brain was also investigated using polymeric poly (n-butylcyanoacrylate) (PBCA) NPs prepared by emulsion polymerization. Tacrine (1 mg/kg) was administered by i.v. injection in the form of a simple solution in phosphate buffered saline; bound to PBCA NPs, and bound to PBCA NPs coated with 1% polysorbate 80(Tween-80) in Healthy adult Wistar rats weighing 180–220 g were obtained from and tacrine concentration was analyzed 1 h post-injection . The brain concentration of i.v. injected tacrine could be enhanced by 4.07-fold as compared to the free drug tacrine after binding to poly (n-butylcyanoacrylate) NPs coated with 1% polysorbate 80[13].

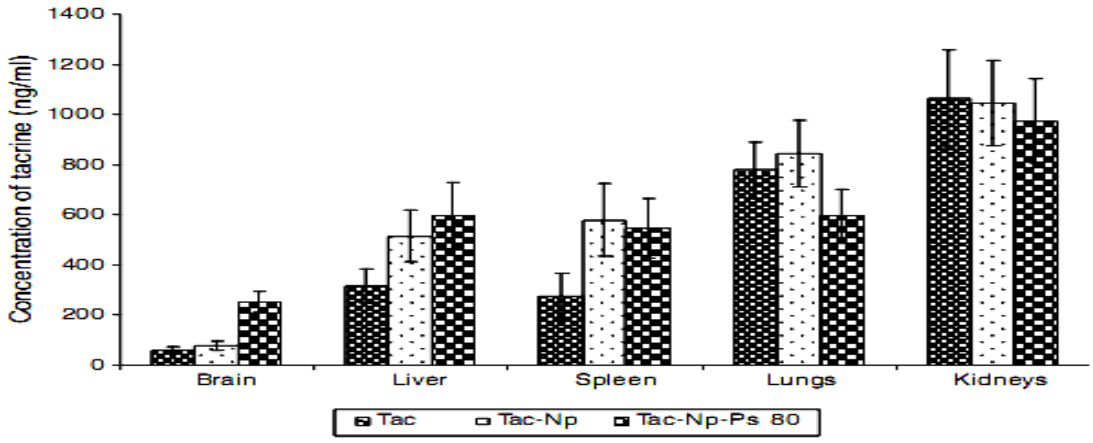

Figure 9: Tacrine concentration(ng/ml) in different organs after Tacrine-loaded PBCA NP's administration

Importance of Polysorbate 80 in Brain Targeting:

Poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80 adsorb apolipoprotein Band/or E after injection into the blood stream. The polysorbate acts mainly as an anchor for the apolipoprotein-overcoated nanoparticles thus would mimic lipoprotein particles and could interact with and then be taken up by brain capillary endothelial cells via receptor-mediated endocytosis. It is possible that uncoated tacrine, rivastigmine loaded nanoparticles indeed were captured by the reticuloendothelial system, and the particles continuously released the drug into the blood stream resulting in elevated plasma and brain levels of tacrine (because tacrine crosses the blood–brain barrier). The developed formulations may also

reduce the total dose required for the therapy with concurrent reduction in dose related toxicity[14]. However, a successful passage of the drug loaded NPs across the BBB is not fully predictive of its therapeutic effect, because after penetration of the drug across the BBB it is equally important to evaluate whether its biological activity is retained or not.

4.7.2 EGCG Solid Lipid Nanoparticles

SLN's (Nano epi gallo catechin 3-gallate) differ from traditional liposomes because they do not require micelle formation. Rather, they are drug: lipid complexes, enables the formation of smaller diameter particles that was hypothesized would be useful for increasing the oral bioavailability of EGCG. Nanolipidic particles (NanoEGCG) were prepared using a proprietary cosolubilization methodology involving use of monophasic liquid preparations.

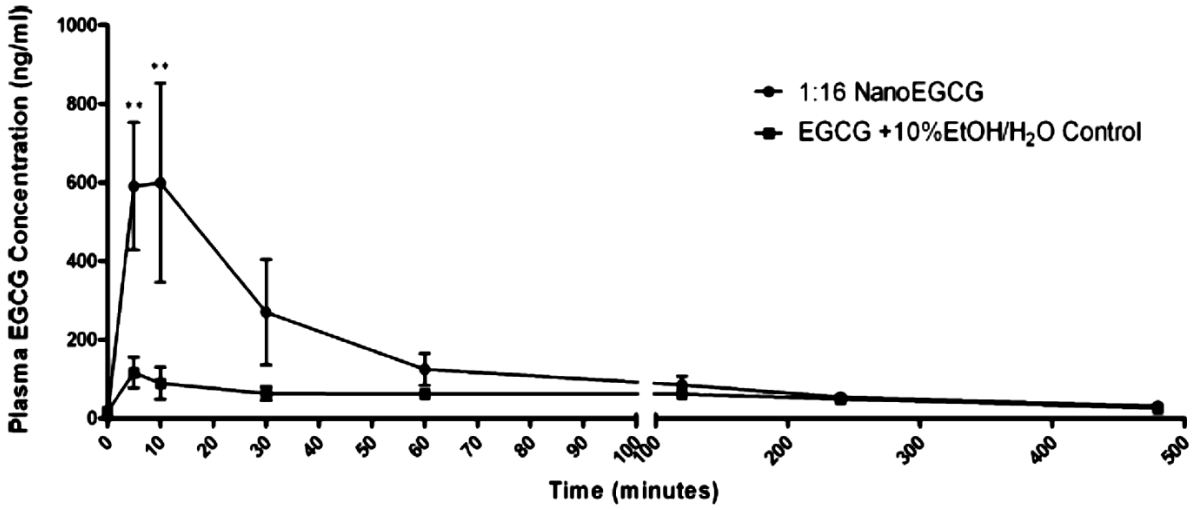

Figure 10: Plasma EGCG conc. After administration of NanoEGCG and EGCG+10% EtOH control

SLN's are highly effective at increasing the absorption of EGCG into systemic circulation. The control was very poorly absorbed in comparison to the NanoEGCG. This study provides important preliminary evidence that nanolipidic particles might be useful for safely translating EGCG into human clinical trials. Not only did NanoEGCG more than

double the oral bioavailability of EGCG in rats but also was more effective at promoting alpha-secretase activity in vitro, even at reduced concentrations. Taken together, it is possible that NanoEGCG will be therapeutically effective at doses that would be considered acceptable in the clinical setting[15].

4.7.3 PEGylated NP

Long-circulating PEGylated polycyanoacrylate nanoparticles act as an adequate vector to target spleen and brain simultaneously. These nanoparticles are formed by an amphiphilic copolymer where the hydrophobic block itself (poly hexadecyl cyanoacrylate, PHDCA) forms the particle core while the hydrophilic part (poly (ethylene glycol), PEG) remains as a surface-exposed 'protective cloud'. Such type of hydrophilic coating reduces the natural blood opsonization process of the particles and, hence, the recognition by macrophages, increasing particles half-life in blood.

4.7.4 Dendrimers

Dendrimers are a new class of polymers which are synthesized in a stepwise manner with branched monomer units. In the first step, branched monomers react with a polyfunctional core, leaving the reactive end groups on the surface. The more layers of monomers are attached, the higher the generation of the dendrimer synthesized[16]. **Polyamidoamine** (PAMAM) dendrimers of the third generation, G3, for example, possess 32 groups on the surface. New nanotherapies have been designed to inhibit the formation of A**B** aggregates during intermediate steps.

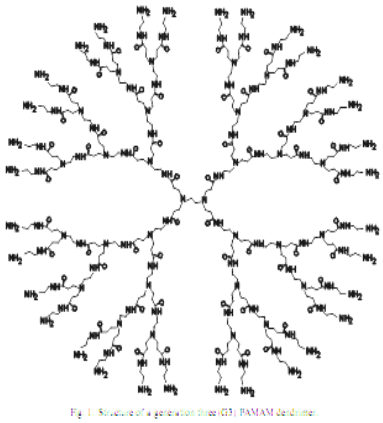

Figure 11: Dendrimer Structure

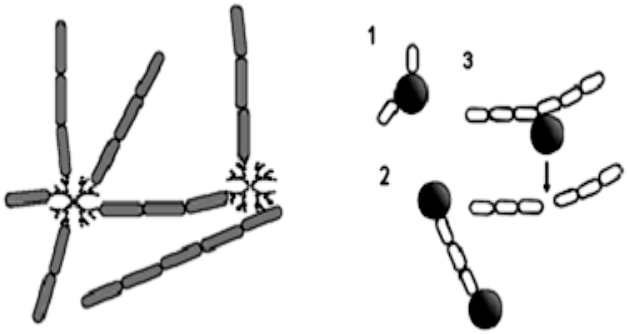

Figure 12 : Dendrimer Action on AB

Polyamidoamine dendrimers inhibit aggregation of AB peptides. Anti-assembly

strategy of dendrimers can be performed either via their binding with peptide monomers or through blocking the end of protofibrils and fibrils. These anti-assembly effects of dendrimers take place at their higher concentrations. Klajnert et al suggested that the higher concentrations of dendrimers will have toxic effect rather than therapeutic results, because in higher concentrations, antiassembly effect of dendrimers happens and prevents AB fibrillization and thereby results in the accumulation of toxic low molecular weight AB oligomers. Low concentrations of dendrimers are supposed to have therapeutic effects, according to their effect on lowering the oligomeric species lifetime. This is because low concentrations of dendrimers induce AB oligomers to form less toxic fibrillar species[17]. The aggregation kinetics of the amyloid peptides were monitored using the dye Thioflavin T (ThT), whose fluorescence is dependent on the formation of amyloid aggregates.

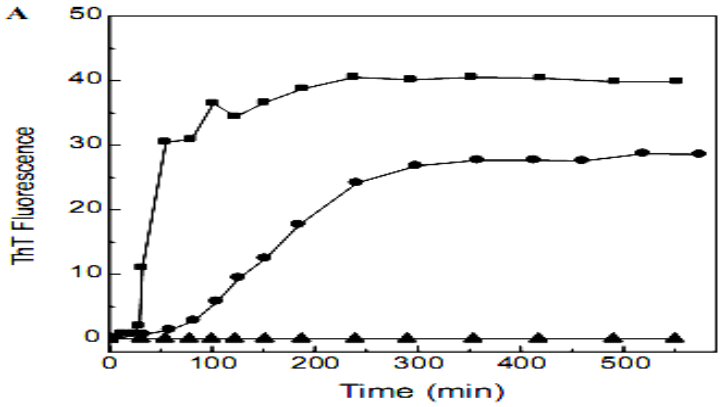

Figure 13: Time dependent Fluorescence variation of ThT

4.7.5 Fullerenes

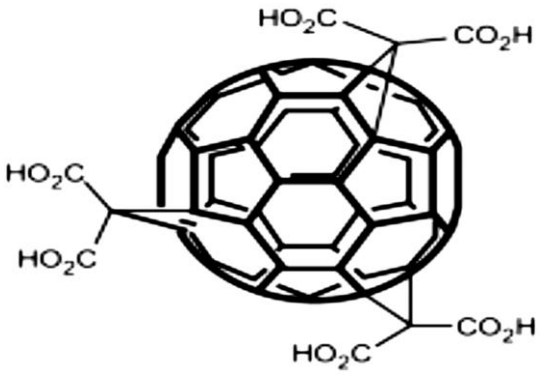

Figure 14: Fullerenes: Structure.

Fullerenes have also been shown to inhibit AB fibrillization. C60 fullerenes affected AB aggregate assembly. Upon intracerebroventricular injection, C60 fullerenes were able to prevent impaired cognitive performance on tasks normally induced by the presence of AB. Presumably, the neuroprotective effect of fullerenols is due to both antioxidant reactions and inhibition of AB42-induced Ca^{2+} neurotoxicity[17]. Huang et al. validated the latter finding in their investigation into the effect of fullerenol-1 upon AB-induced Ca^{2+} influx in the cultured neurons [18]. Dugan et al. have shown that fullerene poses complete neuroprotective properties against NMDA receptor mediated neurotoxicity. NMDA receptor function is important to neuronal mechanisms of learning and memory. Altogether, applications of functionalized fullerene derivatives including carboxyfullerene and hydroxyfullerene (fullerenols), are promising in discovery of new drugs for AD; however further research on their pharmacodynamic and pharmacokinetic properties is necessary[17].

4.7.6 Theranostic Magnetic iron-oxide NPs

A recent example demonstrating this possibility has been the complete removal of amyloidogenic fibrils from an aqueous phase by Binding amyloid fibrils to magnetic iron-oxide (maghemite) NPs and then removing these NP–protein complexes from the solution using a magnetic field[3]. Maghemite NPs nanoparticles have attracted extensive interest due to their super paramagnetic properties and their potential applications in many fields. They are biocompatible and potentially non-toxic to humans. Iron oxide is easily degradable and therefore useful for in vivo applications. The fluorescent maghemite NPs had a combination of the magnetic and fluorescence imaging into one nanostructured system; they had a great advantage as multimodal imaging agents. It was further stated that the hybrid system prepared might enable the early detection of plaques using both magnetic resonance imaging and fluorescence microscopy, and therefore may be applied for in vivo AD diagnosis studies. Besides this, the fluorescent-magnetic NPs can also find useful application as selective biomarkers to detect the location and the removal of amyloid plaques derived from different amyloidogenic proteins that lead to NDs[4].

4.7.7 Theranostics Gold (Au) NP

Theranostics Gold (Au) NPs can target and remove AB deposits with the application of electromagnetic energy. Au NPs

conjugated with fragments of AB peptide or coated with a peptide known to interact with AB aggregates (e.g., CLPFFD-NH_2) can be incorporated into AB fibrils. Stable interaction between Au NP–protein complexes and their target, AB aggregates, is a key goal in the application of this technology. AB aggregates that incorporate Au NPs can be selectively ablated by laser exposure or with the application of microwave fields providing new avenues for both targeting and then removing AB deposits. Microwave radiation is perhaps less invasive while still allowing for selective ablation of AB deposits. These provide new avenues for both targeting and then removing AB deposits[3].The thermal energy was produced from a low gigahertz electromagnetic energy source (microwave) by the gold nanoparticles, which are already attached to the specific target (i.e. AB). Gold nanoparticles are selected for this experiment because of their nanometric size, high surface-to-volume ratio, biocompatibility, high electron density and mobility. These properties make it feasible to provide a specific bond target with a selective supply of energy in a remotely controlled manner, and without any adverse effects on the molecular proximity[17].

4.7.8 Diamondoids

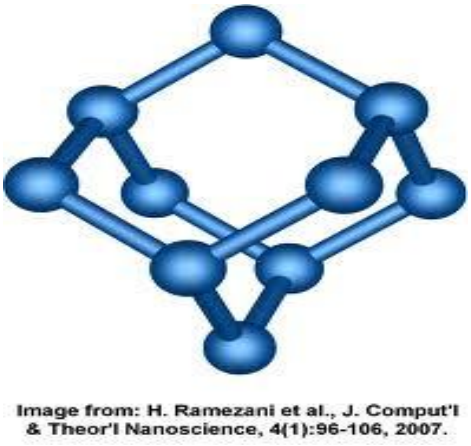

Figure 15: Diamondoid structure

In the context of classical chemistry, "diamondoid" refers to variants of the carbon cage molecule known as adamantane ($C_{10}H_{16}$), the smallest unit cage structure of the diamond crystal lattice. Interestingly, Memantine, a FDA approved neuroprotective drug against AD pathogenesis is a derivative of adamantine (1-amino-3, 5-dimethyladamantane), which is a diamondoid**.** Diamondoids are cage like saturated hydrocarbons, known as one of the nanotechnology molecular building [17].

4.7.9 CHP Nanogels

Biocompatible nanogels composed of a polysaccharide pullulan backbone with hydrophobic cholesterol moieties (cholesterol-bearing pullulan, CHP) as

artificial chaperones to inhibit the formation of $Ab_{1–42}$ fibrils with marked amyloidgenic activity and cytotoxicity. The CHP-nanogels incorporated up to 6–8 $AB_{(1–42)}$ molecules per particle and induced a change in the conformation of AB from a random coil to a-helix- or b-sheet-rich structure. This structure was stable even after 24-h incubation at 37 degree C and the aggregation of $AB_{1–42}$ was suppressed. Nanogels composed of amino-group-modified CHP ($CHPNH_2$) with positive charges under physiological conditions had a greater inhibitory effect than CHP-nanogels, suggesting the importance of electrostatic interactions between $CHPNH_2$ and AB for inhibiting the formation of fibrils. Cell viability was estimated by the 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The absorbance of the cells that had been treated only by the vehicle was set to 100% viability.

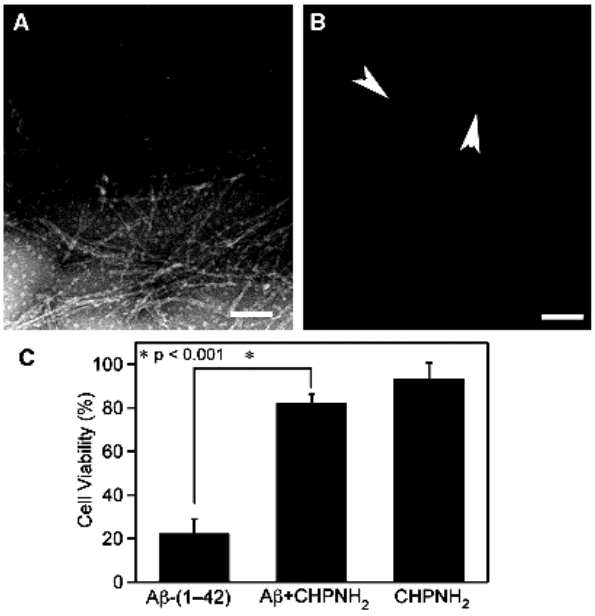

Fig. 5. TEM images of 24-h incubated Aβ-(1–42) (15 μM) without (A) or with (B) $CHPNH_2$ nanogels at a glucose-to-Aβ ratio of 4000 (scale bar = 100 nm). Allow heads show nanogel particles. (C) Cytotoxicity of 24-h incubated Aβ-(1–42) against PC12 cells as determined by the MTT assay (n = 6, ±S.E.). The final concentrations of Aβ and $CHPNH_2$ nanogels were 1 μM and 4 mM (as glucose), respectively.

Figure 16: TEM images of AB_{1-42} without(A) or with(B) $CPNH_{2\ NP.}$ C shows cell viability

The cell viability was decreased to 22%. The co-existence of $CHPNH_2$ nanogels significantly reduced the toxicity of AB and recovered the viability to 82% at a glucose-to-protein ratio of 4000. Furthermore, it should be noted that nanogels themselves had no harmful effects. Therefore, smaller amounts of nanogels appear to sequester several AB

molecules in a particle, imposing a non-fibrillar b-sheet conformation and inhibiting the formation of fibrils. The spectrum of AB without a nanogel changed from that of a random coil to one of a b-sheet-rich structure accompanied by an increase in ThT fluorescence after a 24-h incubation at 37degree C. In the presence of large amounts of nanogels, where each particle contains one protein molecule, $AB_{1–42}$ maintained an a-helical structure and exhibited less than 15% ThT fluorescence even after a 24-h incubation, suggesting that the nanogels effectively suppressed the aggregation of AB $_{(1–42)}$. Moreover, with the addition of MbCD(methyl-b cyclo dextrin), the trapped AB is released as a monomer without the formation of putative toxic oligomers. Furthermore, $CHPNH_2$ nanogels have greater cellular up-take efficiency than the cationic liposomes widely used in drug delivery systems Another advantage of nanogels is that they can control the conformation of AB .Therefore, nanogel-AB complexes are applicable for conformation-specific vaccination[19].

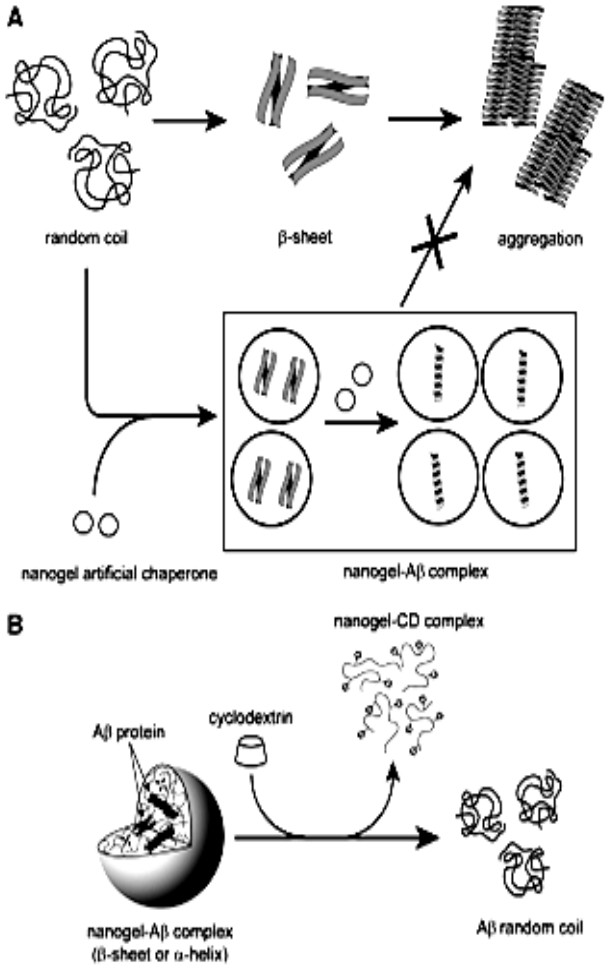

Figure 17: Mechanism of Action of Nanogels

Over the last decade diagnostic and therapeutic potential of NPs for the AD have been extensively investigated. Although experimental data have demonstrated effective transport of drugs across BBB using NPs for the treatment of AD, still there is a need to optimize this general strategy, in terms of efficiency, specificity and safety. One should be cautious because a successful passage of drug loaded nanoparticulate delivery system across a simulated BBB model is not fully predictive of its therapeutic effect, because after penetration of the drug across the BBB it is equally important to evaluate whether its biological activity is retained or not. Safety and toxicity aspects of the NPs are important considerations that need to be taken seriously, understood and resolved before the extensive clinical use of these formulations for the treatment of AD.

4.8 Intranasal Mucoadhesive Microemulsion of Tacrine

Mucoadhesive drug delivery systems are those that provide intimate contact of the drug are those that provide intimate contact of the drug with the mucosa for an extended period of time. Since the nasal mucosa offers numerous benefits as a target tissue for drug delivery, a wide variety of therapeutic compounds may be administered intranasally for topical, systemic and central nervous system action. The use of mucoadhesive system as microspheres is to provide a drug protection from enzymatic degradation and thus increase the contact time with the nasal mucosa. These Intranasal (IN) microspheres can be efficiently utilized to avoid hepatic first-pass metabolism, improve therapeutic efficacy and enhance residence time on nasal mucosa. The gamma scintigraphy images clearly demonstrate the accumulation of formulations in brain at 15 minutes post dosing when administered via IN routes. However, after IV administration very little or no accumulation of radioactive formulation was observed. This suggested selective nose-to-brain direct transport of drug. The accumulation of higher radioactivity in the brain after intranasal administration of TMME compared with TS and TME demonstrates the role of mucoadhesive microemulsion in brain targeting. Thus after nasal administration of different tacrine formulations, rapid delivery of tacrine to the brain compared with IV administration may be because of preferential nose-to-brain transport after IN administration. This was further supported by the lower Tmax values for brain

compared with blood for all the 3 nasally administered formulations. Under normal circumstances, nasally administered formulations get cleared quickly from the nasal cavity due to mucociliary clearance[20]. Mucoadhesive agents are well reported to prolong the contact time of the formulation with the nasal mucosa and thereby enhance rate and extent of absorption of the drug[21].

4.9 The Rivastigmine Transdermal Patch (Exelon)

A transdermal drug delivery system has the potential to change the treatment paradigm for many AD patients.

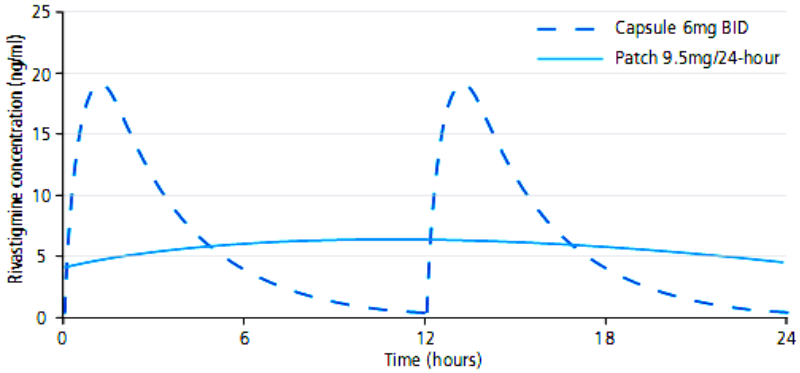

Figure 18: Rivastigmine TD Patches in comparison to Rivastigmine Capsules

Cholinesterase inhibitors have been shown to exhibit dose–response relationships with higher plasma levels of the drug corresponding to higher levels of enzyme inhibition. However, the incidence of adverse events (AEs) also increases with higher oral doses, particularly gastrointestinal occurrences such as nausea and vomiting. Consequently, not all patients in clinical practice are able to achieve and maintain the recommended therapeutic doses of conventional oral cholinesterase inhibitors. A transdermal patch can provide smooth and continuous delivery of the drug, reducing Cmax and prolonging tmax while maintaining drug exposure. This pharmacokinetic profile has the potential to reduce the incidence of cholinergic side effects, allowing patients easier access to optimum therapeutic doses, thus improving the effectiveness of treatment over oral administration. Additional benefits of transdermal administration, include a simplified treatment regimen, convenience and ease of use[22].

Rivastigmine is chemically well-suited to transdermal delivery. The rivastigmine patch uses modern matrix technology, combining the drug, antioxidants, a polymer mixture (to control the drug delivery rate) and a silicon matrix adhesive into a single layer through which the drug diffuses. Unlike early transdermal patches, there is no 'reservoir' of the drug within the patch or adjunct (such as ethanol) to facilitate diffusion of rivastigmine through the skin.

5. FUTURE:

Three important avenues for disease-combating interventions will be developed through nanomedicine approaches. First, the improvement of site-directed drug delivery in brain regions most affected by disease will be achieved through **'smart formulations'** and the ability to bypass or engage the BBB, thus improving the outcomes of therapeutic approaches. Second, regenerative nanomedicine will provide new agents to specifically repair or modulate disease targets. However, such interventions must not only find their way into affected disease areas of the CNS but also show limited or no toxicities. Third, early disease diagnosis will lead to improved intervention outcomes since treatments are likely to be more effective. Interdictive therapies that can halt or reverse the disease course have been tried but were met with varied degrees of success.

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