Chapter 3:

Drug and Excipient Profile

3 Drug and Excipient Profile

3.1 Memantine

Memantine is an FDA approved drug used for the management of Alzheimer's Disease (AD). It is an N-methyl-D-aspartate (NMDA) receptor antagonist that acts by a different mechanism unlike the cholinesterase enzyme inhibitors normally employed in the management of Alzheimer's disease. The chemical name of Memantine hydrochloride is 1-amino-3, 5-dimethyl adamantine hydrochloride with molecular formula $C_{12}H_{21}N$•HCl, and molecular weight 215.76. With structure as shown in figure 3, Memantine HCl occurs as a fine white to off-white powder and is soluble in water [162,163].

Figure 3: Structure of Memantine.

3.1.1 Dosage and Administration

The initial recommended dose of NAMENDA is 5 mg once daily but the dose can be increased from 5mg to 10 mg/day (5 mg twice daily), 15 mg/day (5 mg and 10 mg as separate doses), and 20 mg/day (10 mg twice daily) for desired therapeutic effects [163].

3.1.2 Mechanism of action of Memantine

This drug inhibits calcium influx into cells which normally occurs by chronic NMDA receptor activation by glutamate (Figure 4). Memantine blocks the effects of glutamate, a neurotransmitter in the brain that leads to neuronal excitability and excessive stimulation in Alzheimer's Disease which leads to improvement of symptoms of dementia, demonstrated by increased cognition and other beneficial central nervous system effects. According to the reported studies and clinical trials lower concentrations of Memantine are normally used to enhance neuronal synaptic plasticity in the brain to improve memory and act as a neuroprotectant against destruction of neurons caused by excitatory neurotransmitters. A higher dose of NMDA receptor antagonist showed improved neuronal synaptic plasticity which involved improvement in learning and memory retention process. Memantine exhibits minimal activity for GABA, benzodiazepines dopamine, adrenergic, histamine, and glycine receptors, as well as voltage-dependent Ca^{2+}, Na^{+}, or K^{+} channels but has antagonist activity at 5HT3 receptors. The overstimulation of neurons due to excitatory properties of glutamate contribute to neurotoxicity [164].

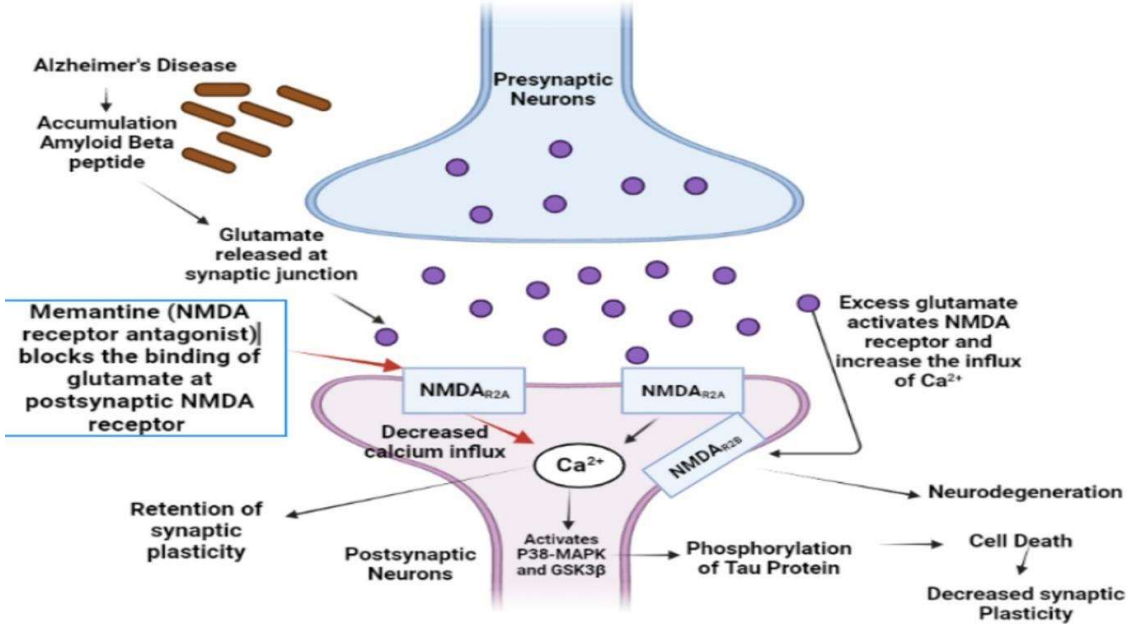

Figure 4: Glutaminergic pathway and mechanism of action of Memantine.

3.1.3 Pharmacokinetics

Orally administrated Memantine shows high absorption of drug with maximum peak concentrations in about 3-7 hours with linear pharmacokinetics over the therapeutic dose range. The mean volume of distribution of Memantine is 9-11 L/kg and the plasma protein binding is low (45%). Memantine undergoes partial hepatic metabolism and the hepatic microsomal CYP450 enzyme system does not play a significant role in the metabolism of Memantine. Memantine is predominately excreted (about 48%) in unchanged form in urine with an elimination half-life of about 60-80 hours. The rest of Memantine is converted primarily to three polar metabolites which possess minimal NMDA receptor antagonistic activity: the N-glucuronide conjugate, 6-hydroxy memantine, and 1-nitroso deaminated memantine. A total of 74% of the administered dose is excreted as the sum of the parent drug and the N-glucuronide conjugate. The renal clearance of Memantine involves active tubular secretion moderated by pH-dependent tubular reabsorption [165,166]. The dose adjustment is not recommended for patients with mild and moderate hepatic impairment. Memantine should be administered with caution to patients with severe hepatic impairment as the pharmacokinetics of memantine has not been evaluated in that population [167,168]. Co-administration with cholinesterase inhibitors and AChE inhibitor donepezil HCl did not affect the pharmacokinetics of either compound. Furthermore, Memantine did not affect AChE inhibition by donepezil [167,169]. *In vitro* studies conducted with marker substrates of CYP450 enzymes (CYP1A2, -2A6, -2C9, 2D6, -2E1, -3A4) showed minimal inhibition of these enzymes by Memantine. In addition, it

also indicates that at elevated concentrations , Memantine does not induce the cytochrome P450 isozymes CYP1A2, -2C9, -2E1, and -3A4/5 [163].

3.2 PLGA

PLGA (poly (lactic-*co*-glycolic acid) is a copolymer with a weight-average molecular weight of about 80000 g/mol, approved by the Food and Drug Administration (FDA) for therapeutic use. The ratio of lactide to glycolide used for the polymerization determines the crystallinity of PLGAs, which varies from fully amorphous to fully crystalline types. These properties also affect the ability to be formulated as a drug delivery device and may control the device degradation rate and hydrolysis. Mechanical strength, swelling behaviour, capacity to undergo hydrolysis and subsequently biodegradation rate of the polymer are directly influenced by the degree of crystallinity of PLGA, which is further dependent on the type and molar ratio of the individual monomer components in the copolymer chain. Crystalline PGA, when co-polymerized with PLA, reduces the degree of crystallinity of PLGA and as a result increases the rate of hydration and hydrolysis.

PLGAs typically shows a glass transition temperature in the range of 40-60 °C. PLGA can be dissolved in a wide range of solvents, depending on composition. Higher lactide polymers can be dissolved using chlorinated solvents whereas higher glycolide materials will require the use of fluorinated solvents. The hydrolysis of PLGA occurs due to the cleavage of its ester linkages in presence of water and the time required for its degradation depends on the monomer ratio: the higher the content of glycolide units, the lower the time required for degradation as compared to lactide polymers [170]. In addition, polymers that are end-capped with esters (as opposed to the free carboxylic acid) demonstrate longer degradation half-lives. The tuneable pattern of structural and functional properties makes it convenient for the fabrication of many medical devices, such as grafts, sutures, implants, prosthetic devices, surgical sealant films, micro, and nanoparticles. The hydrolysis of PLGA in the body produces monomers of lactic acid and glycolic acid which enter various metabolic pathways. Lactic acid is metabolized in the tricarboxylic acid cycle and eliminated as carbon dioxide and water and Glycolic acid is metabolized in the same way, and excreted through the kidney [171]. However, it has been reported that the acidic degradation of PLGA reduces the pH at the local site to create an autocatalytic environment [172,173].

Biodistribution and pharmacokinetics of PLGA follow a non-linear and dose-dependent pattern. Earlier studies have reported that clearance of PLGA from circulation and uptake by the mononuclear phagocyte system (MPS) may depend on dose and composition of PLGA carrier

systems [174]. Furthermore, some formulations of PLGA, such as nanoparticles, accumulate rapidly in liver, bone marrow, lymph nodes, spleen and peritoneal macrophages. However, PLGA hydrolyses into glycolic acid and lactic acid and 30% of hydrolysed PLGA is removed by respiration in the lung through kerbs and glycolic acid cycle [175,176].. To address these limitations, studies have investigated the role of surface modification, suggesting that incorporation of surface modifying agents can significantly increase blood circulation half-life [177].

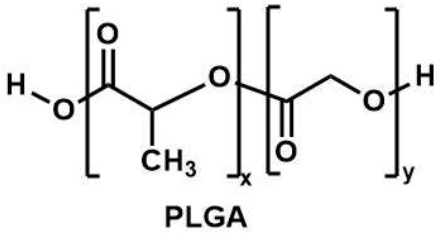

Figure 5: Structure of PLGA where x is number of units of lactic acid; y is number of units of glycolic acid.

PLGA (50:50) is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA) in 50: 50 ratio with structural formula $C_5H_8O_5$, molecular weight 10000-20000 g/mol and transition temperature 43°C which can be used to fabricate devices for drug delivery and tissue engineering applications (Figure 5) [178]. PLGA (75:25) is an amorphous solid with structural formula $C_5H_8O_5$ and molecular weight 66,000-107,000 g/mol with a transition temperature (Tg) in the range of 45-50°C. It is soluble in most organic solvents such as methylene chloride, tetrahydrofuran, ethyl acetate, acetone, chloroform, and hexa fluoro-ispropanol.

3.3 Pluronics F-127

Pluronics F-127 also known as Poloxamer 407, is a hydrophilic non-ionic surfactant that is a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol (PEG) (PEO-PPO-PEO) of general formula $C_7H_{16}O_4$, with an average molar mass of 12600 g/mol (Figure 6). The approximate lengths of the two PEG blocks are 101 repeat units, while the approximate length of the propylene glycol block is 56 repeat units

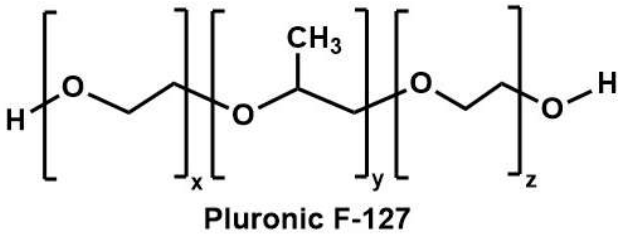

Figure 6: Structure of Pluronics F-127 (PEO-PPO-PEO).

Pluronics F-127 has unique ability to self-assemble its copolymer into micelles with a hydrophobic PPO centre core and a hydrophilic PEO outer shell that interfaces with water in aqueous environment. Although Pluronics F-127 is often described as the least toxic commercially available copolymers with lethal dose concentration of LD_{50}>5000 mg/kg [149]. Consequently, these self-assembling molecules can be effectively used as drug carriers to deliver drugs into subcellular compartments by slowly releasing hydrophilic–hydrophobic encapsulated excipients into physiological fluids. In addition, the sol-gel transition is governed by the composition, molecular weight, and concentration of each constituent block polymer. Self-assembly is a temperature or pH-dependent phenomenon and affects the degradation properties of the biomaterial: below a certain temperature or pH both the ethylene and propylene oxide blocks of Pluronics F-127 are hydrated and the PPO block becomes soluble but above certain temperature or pH it shows self-assembly [179]. Pluronics F-127 forms a thermo reversible gel that is used for administration of many types of drugs through various routes of administration including oral, topical, intranasal, vaginal, rectal, ocular, and parenteral routes [180]. Pluronics F127 has shown enhancement in the therapeutic efficacy and thermo stability when loaded with various therapeutic proteins for brain disorders and tumour-targeted delivery. Similarly, Pluronic F127 has also shown to improve the pharmacokinetic and pharmacodynamic parameters of incorporated drugs by improvement in the oral bioavailability, especially poorly water-soluble drugs. Pluronic F127 in combination with other polymer additives showed increased thermostability, prolonged therapeutic efficacy via sustained release phenomenon, and displayed minimal toxicity at injection site. There have been some reports on in vivo fate of poloxamer polymers. The pharmacokinetics and tissue distribution of Pluronic F-127 in rats after 5mg/kg *i.v* injection (Feng *et al*., 2021) exhibited $AUC_{0\text{-}t}$ of 3.0 ± 0.6μg/L*h, MRT of 0.6±0.1h, Clearance of 1.7±0.3 L/h/kg, $t_{1/2}$ of 2.0 ± 1.1h; and V_d of 5.1± 3.2 L/kg. The pharmacokinetic and biodistribution study indicated rapid distribution of Pluronic F-127 to tissues with a high clearance rate. Higher distribution of Pluronic F-127 were obtained in kidney (26.8μg/g at 0.2h and 11.63μg/g at 4h) followed by other tissues such as stomach, liver, lung, muscle and spleen [181,182].

3.4 Polyethylene Glycol (PEG)

PEG is also known as polyethylene oxide (PEO) or polyoxyethylene (POE) with chemical formula $C_{2x}H_{4x+2}O_{x+1}$ and molecular weight of 44.05x + 18.02 g/mol where x denotes the average number of oxyethylene groups (Figure 7). PEG is considered biologically inert and safe by the FDA. PEG is uncharged, water soluble, nonreactive agent without any specific

interaction with receptors in the body**.** Pharmaceutical-grade PEG is used as an excipient in many pharmaceutical products, in oral, topical, and parenteral dosage forms. However, PEG can be modified and crosslinked into a hydrogel and used to mimic the extracellular matrix (ECM) environment for cell encapsulation. PEG is soluble in water and is minimally absorbed in the gastrointestinal tract and forms hydrogen bonds with water molecules [183]. The PEGylation technology has been widely used to modify biopharmaceutical properties of nanocarriers, to decrease their in vivo clearance and also potentially to ameliorate the immunogenicity of highly immunogenic drugs, cells and proteins such as enzymes. Oxidation of terminal hydroxyl to carboxyl groups of PEGs occurs by alcohol dehydrogenase and cytochrome P450 isoenzymes [184]. Oral absorption and metabolism of PEG in human volunteers decreases with increasing MW or at higher chain lengths. 57% absolute bioavailability was observed for PEG500 and 9.8% for PEG1000, was not quantifiable for PEG6000. However, urinary excretion after intravenous administration (1 g) increased from 77% for PEG300 to over 85% for PEG1000 and to 96% for PEG6000 within 12 hours post dose. The elimination of PEG obtained via urinary excretion and half-lives calculated from these early studies suggested excretion half-life of 1 hour for PEG300, 1.8 hour for PEG1000 and 4–7 hours in PEG3350 in plasma; and, faecal excretion after oral administration was 93% (Figure 8). The renal clearance is main elimination pathway for high MW PEGs and rate is dependent on the total MW of PEG, the length of PEG chains and, in the case of PEGylated nanocarriers, the overall MW of the product [185].

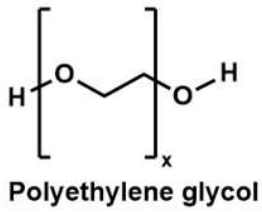

Figure 7: Structure of Polyethylene glycol where x denotes the average number of oxyethylene groups.

PEG coating to nanocarriers imparts shielding effect to the surface which prevents aggregation, opsonisation, and phagocytosis of nanocarriers and prolongs its systemic circulation in body. PEG coatings on nanocarriers have also been utilized for efficient drug and gene delivery by overcoming various biological barriers associated with modes of administration like that of intrathecal, ocular and intranasal [186]**.**

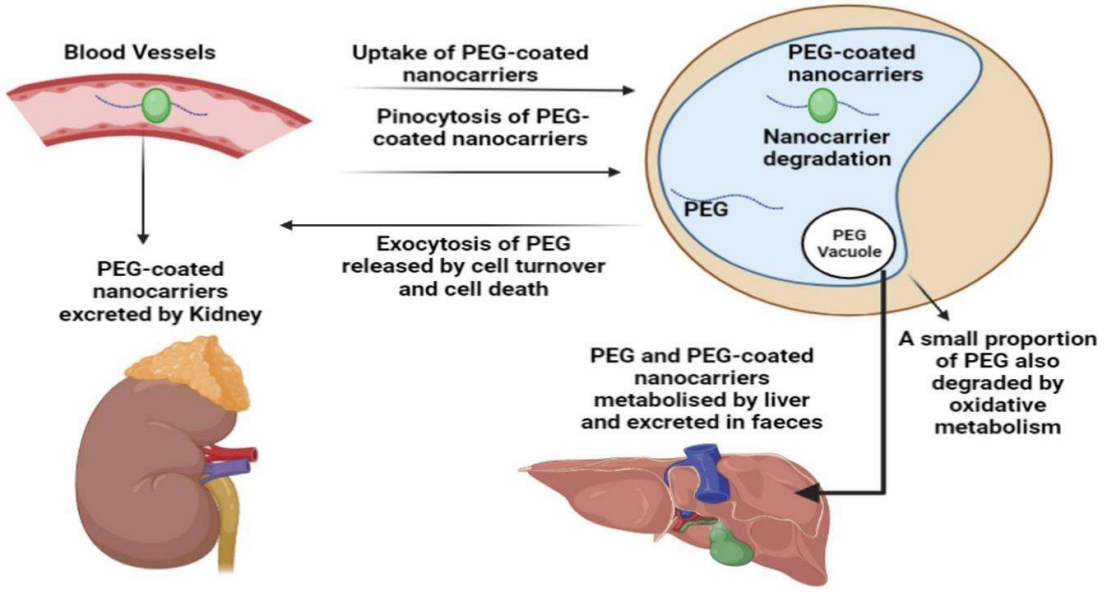

Figure 8: Metabolism, degradation and Excretion of PEG and PEG-coated nanocarriers [187]

3.5 Bone Marrow stem cell (BMSCs)

Murine BMSCs which are supplied in cryovials, after thawing should be maintained in complete growth media (Mesenchymal Stem Cell Growth Medium (MSCGM)) following the detailed protocol described below [188]. Murine BMSCs are generally isolated from tibia and femoral bones and cultured in medium with Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) for 3 h at 37ºC and 5% CO2 in an incubator. After replacement with fresh media, nonadherent cells are removed after 3h. After achieving confluency of primary culture, the culture is treated with 0.5 ml of 0.25% trypsin containing 0.02% ethylenediaminetetraacetic acid for 2 min at room temperature (25ºC). A purified population of BMSCs can be obtained 3 weeks after the initiation of culture [189,190]. BMSCs has unique ability to develop into different cells including osteoblasts, adipocytes, chondrocytes and hematopoiesis-supportive stroma. The cells are mobilized to other tissues, to sites of inflammation, such as areas of injury and tumours and respond to the local microenvironment, and exert immunosuppressive and anti-inflammatory activities [191]. Recent studies reported the generation of extracellular matrix and enhanced their potency upon stem cell-based therapy [192]. Transplanted stem cells stimulate the release of cytokines, growth factors and extracellular matrix (ECM) molecules that act either on themselves (autocrine actions) or on neighbouring cells (paracrine actions) (Figure 9). Stem cells also prevent damage from oxygen free radicle by producing antioxidants and anti-apoptotic molecules. In addition, some angiogenic factors, antifibrotic factors, are also secreted by stem cells which are responsible for ECM homeostasis and neuroregeneration [193]. Therefore, novel

research techniques aspire to use stem cells as drug delivery vehicles to contribute as molecular cues to facilitate neuroregeneration in various neurodegenerative disorders.

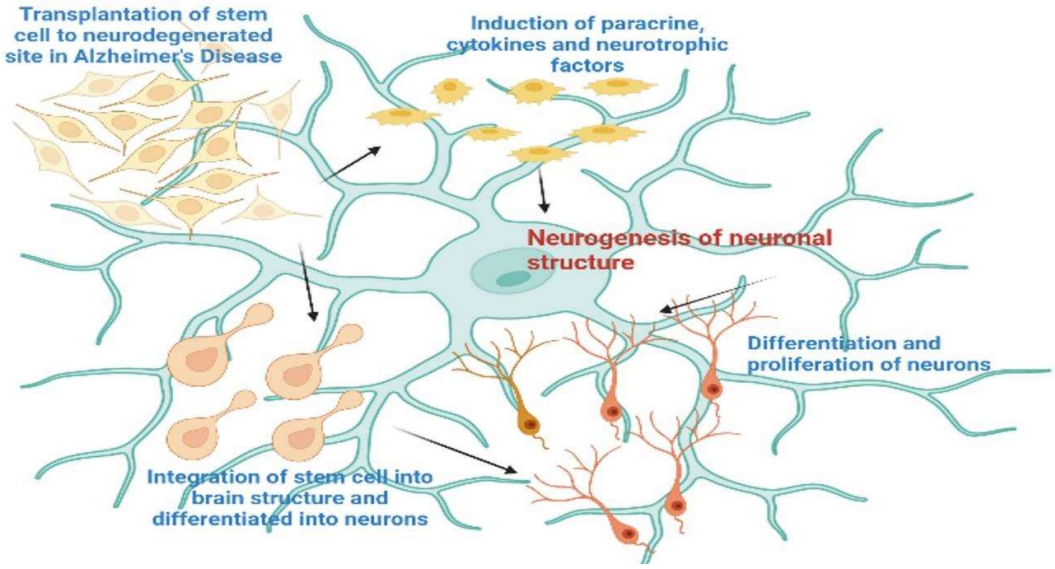

Figure 9: Neurogenesis mediated via stem cells to the site of neurodegeneration.