Chapter 2

Drug repurposing in Alzheimer's Disease

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Literature review, hypothesis and work plan:

2.1 Formulation of system analysis: Hepatobilliary-Enterohepatic circulation of amyloid-beta

Amyloid-beta from brain is transported via liver to bile (biliary clearance of amyloid-beta), and this bile drains into small intestine with amyloid-beta. Hence, there is appreciable amyloid-beta content in the feces. When the feces move to the ileocaecal region, the most majority of amyloid-beta is absorbed into blood of the portal vein and goes to liver circulation. The result is that there is very little amyloid-beta in stool and large intestine. In the ileocaecal region, besides amyloid-beta, other metabolites and valuable substances such as bile salts, biliary lipids, estrone, folic acid, vitamin B12, and urobilinogen are also absorbed into the blood, transiting to the liver. Our aforesaid proposition is illustrated in Figure 2, which we have constructed using data from Ghiso et al. (2004). We can take that amyloid-beta undergoes circulation via hepatobiliary excretion and then enterohepatic uptake from a systems analysis aspect.

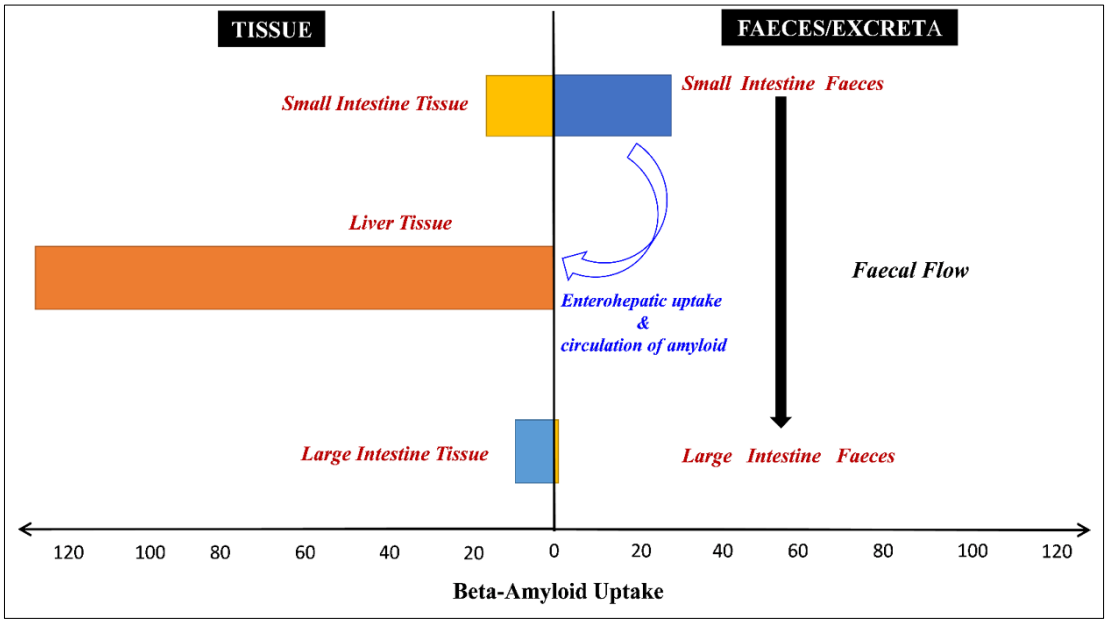

Figure 2: *Depiction of evidence of Aβ undergoing enterohepatic circulation.*

2.2 Receptors implicated in the hepatobiliary - enterohepatic circulation of Aβ

2.2.1 ABCG2 and ABCB1/MDR1 receptor

The ATP-binding cassette (ABC) transporters are a family of membrane-bound protein pumps, which utilize the hydrolysis of ATP to transport compounds across membranes. It is encoded with 49 genes and divided into A to G of 7 subfamilies. Particularly, the transporters ABCG2 and ABCB1 (Multidrug-resistant protein 1) are the proteins highly expressed in the blood-brain barrier and help in the efflux of Aβ from brain tissue into blood [38]. These receptors are also expressed throughout the gastrointestinal tract including liver, and increasing the expression of these receptors can promote Aβ clearance [39]. It may be mentioned that rifampicin is a PXR (Pregnane X Receptor) agonist and induces the expression of ABCG2 and ABCB1/MDR1 proteins (Figure 3) by activating the nuclear receptor [40]. Hence, this repurposed drug can stimulate the clearance of Aβ.

2.2.2 Liver X Receptor-beta

Amyloid beta is hypothesised to be produced from the amyloid precursor protein (APP) in membrane rafts that are rich in cholesterol; cellular cholesterol deprivation inhibits the production of Aβ. The liver X receptors (LXR), which are widely expressed in cells of the central nervous system, are essential regulators of genes that control cellular cholesterol efflux and membrane composition. Sun el al. showed that treating APP-expressing cells with LXR activators reduces the formation of Aβ. LXR activation increases the levels of the ATP-binding cassette transporter A1 (ABCA1) and stearoyl CoA desaturase (Figure 3), and expression of these genes individually decreases the formation of Aβ [41]. Moreover, activation of Liver X Receptor-β (LXR-β) via therapeutic agents like 24-hydroxycholesterol, metformin, digoxin, etc [42] promotes the expression of apolipoprotein E in the liver. The human apoE protein is a 299 amino acid glycoprotein

with variable levels of post-translational sialylation through O-linked glycosylation at the threonine 194 residue [43]. It has also been demonstrated that lapidated apoE facilitates the degradation and clearance of soluble species of Aβ from the brain, using the neprilysin (NEP) in the endolytic compartment of microglia and extracellularly through the actions of insulin-degrading enzyme (IDE). ApoE is expressed in several organs with the highest expression in the liver, followed by the brain [44]. Therefore, these agents may be helpful to degrade the Aβ, which undergoes enterohepatic circulation. Additionally, aphosphodiesterase 3 (PDE3) inhibitor, Cilostazol, also increases ABCA1 expression (Figure 3) [45]. Hence, the aforesaid pharmacological agents may be investigated as anti-Aβ intervention.

2.2.3 Apical sodium-dependent bile acid transporter (ASBT) receptor

Terminal ileal enterocyte is responsible for reabsorption of various valuable substances like bile salts, biliary lipids, biliary phospholipids, estrone, estriol, folic acid, vitamin B12, and urobilinogen, whose loss would be disadvantageous to the organism. Apical sodium-dependent bile acid transporter (ASBT) acts as an effective bile acid transporter system in the ileal enterocyte, which helps to carry bile acid across the brush border of the ileal enterocyte [46]. According to our approach, Aβ along with bile acids, can also undergo reuptake by the ASBT receptor. Inhibiting this receptor can play an essential role in preventing the reabsorption of Aβ through apical sodium transporter. Our next therapeutic agent, resveratrol, promotes the degradation of the human bile acid transporter (ASBT) (Figure 3) [47]. Previous studies show the crucial role of resveratrol in Aβ clearance by different pathways [48].

2.2.4 Bile Salt Export Pump (BSEP) receptor

The anti-diabetic drug metformin increases the expression of Bile Salt Export Pump (BSEP) receptor encoded with the ABCB11 gene (Figure 3), thus promoting more biliary

clearance of Aβ [48]. Enhancing the systemic elimination of Aβ through biliary clearance can reduce its burden in the brain [10]. Altered bile acid profile plays an important role in Alzheimer's disease [49]. Indeed, Type 2 diabetes mellitus (T2D) and hyperinsulinemia are the predominant risk factors in AD which is sometimes integrated with Type 3 diabetes. Metformin is a safe, inexpensive medicine proven efficacious in treating T2D by reducing peripheral blood glucose and synergizing insulin levels [50].

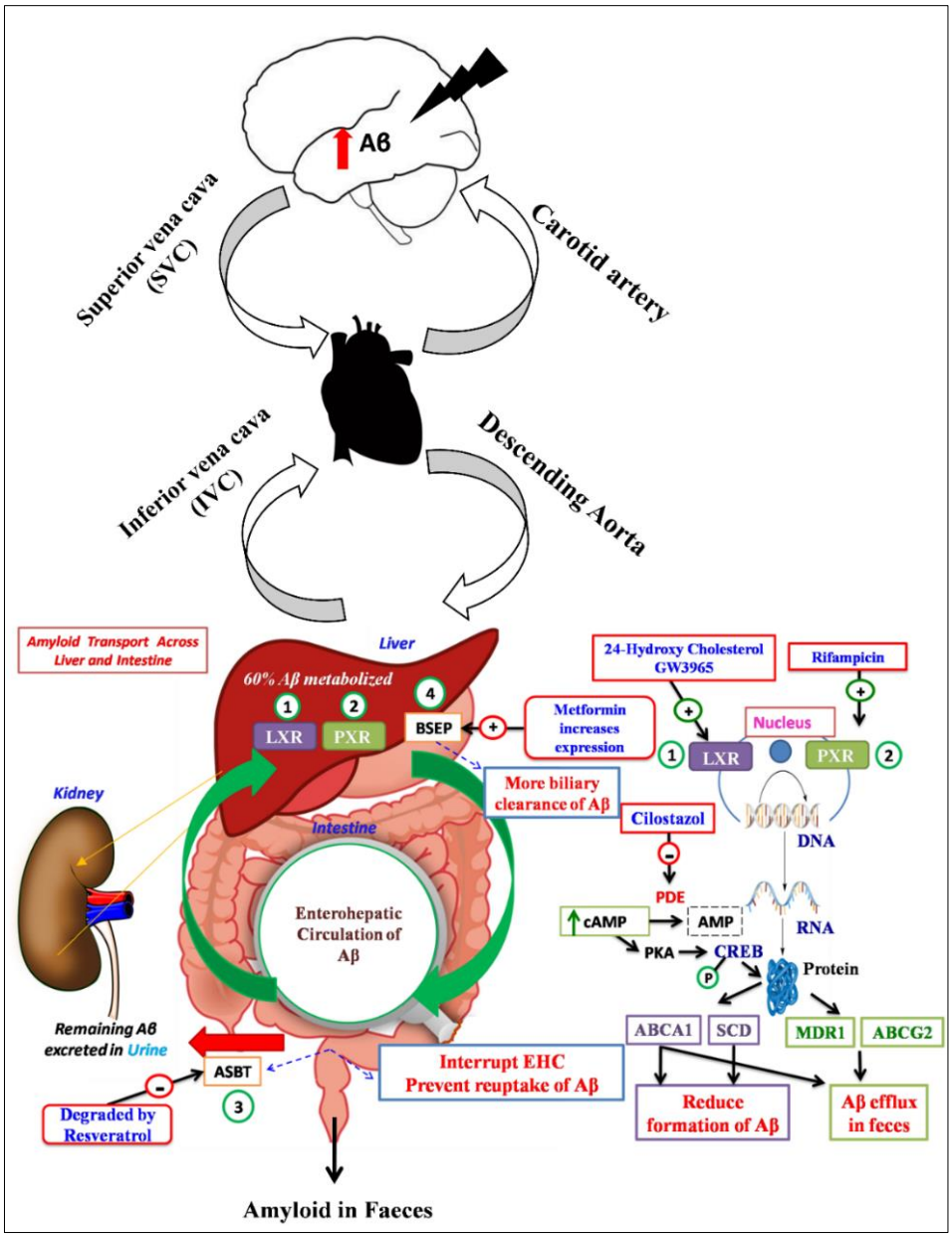

Figure 3: *Depiction of the mechanism of action of the receptors to promote the Aβ clearance from the body as well as the therapeutic agents that may be used to increase the expression of those receptors.*

2.3 Framework of the investigation

We have performed a docking study and molecular dynamics analysis to assess the affinity of amyloid-beta and the proposed drugs targeting the respective receptors. We have implemented the network pharmacology and gene ontology analysis to test whether treatment with specific receptor agonists (similar to our proposed drugs) increases the expression of target genes which helps in the efflux and clearance of amyloid-beta across the liver and intestine, into the faeces. We have also conducted Ingenuity Pathway Analysis to validate our approach. Afterward, we utilized pharmacological synergism analysis to optimize the performance of combination therapy to identify the best possible combination of repurposed drugs to enhance the overall amyloid-beta clearance from the body through (i) maximization of hepato-biliary elimination of amyloid-beta and (ii) minimization of intestinal reabsorption of amyloid-beta.