Chapter 10

Summary and Conclusion

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10.1 Summary of major findings

Our investigation has identified FDA-approved drugs as potential candidates for neuropharmacological repurposing since we have found that there is appreciable evidence to indicate that these therapies may be effective in neuroprotection, which is urgently needed for a therapeutic approach in Alzheimer's disease (AD) and in Neurotropic Viral Infections as Japanese encephalitis and SARS-COV2.

The issue of ageing induced dysfunctionality and neurodegenerative disease, is an impending demographic dilemma worldwide, and particularly for Alzheimer's disease and dementia, as the majority of clinical trials are not much encouraging. Across the last twenty years, there has been only a very few new robust pharmacological entities put forward to have substantial clinical impact. Indeed, the socioeconomic disease burden is worldwide, both in global north and south. Particularly this effect is stark in the major Asian regions which would become aged before they become affluent. By 2050, about 153 million people [201] would have dementia (roughly the same population of Western Europe today) and the total direct and indirect cost would approach $10 trillion annually, with low and middle income countries bearing $6 trillion of cost. Hence the critical need of a possible approach to dementia therapeutics using affordable repurposing drugs for which clinical safety and tolerance has been well established, and this framework might be an expedient way to address the pressing need for an approach to efficacious dementia treatment.

Recently it has been found that amyloid beta is the main etiopathological factor in AD. In the normal physiological system, there is equilibrium between the amyloid generated in tissue and excreted out via the blood. The majority of this amyloid (about 70%) is metabolized by the liver and thus the amyloid elimination path is Brain →Blood →Liver →Bile →Faeces. Several reports suggest that hepatic dysfunction enhances

amyloid in brain and increases AD incidence [86]. We have shown the critical significance of hepatic clearance of amyloid along with potential therapeutic implication in AD. The amyloid-beta transport through Hepatobiliary-Enterohepatic Circulation (HEC) is a critical contributor to the development of amyloid-beta load in the brain. Our reprofiled pharmaceuticals, namely Rifampicin, Resveratrol, Metformin, and Cilostazol, may be able to target the putative receptors implicated in the aforesaid hepatic amyloid circulation and hence enhance the intestinal amyloid-beta excretion faecally and thus decrease the amyloid-beta reabsorption and accumulation in the brain.

Moreover, our investigation elucidates that the systemic availability of hepatometabolic agents such as cholic acid in the serum might be an imperative factor in the formation of Alzheimer's disease by the amyloid load, and cerebral blood flow in the brain. Indeed, our study shows the occurrence of a chain process, namely decrease of serum choline causatively increases amyloid load in the brain and this rise of amyloid load causatively diminishes cerebral blood flow. Hence, exploring the possibility of therapeutic augmentation of serum cholic acid (or their pharmacological derivatives) may be probed as an investigative approach to the clinical management of AD. Therefore, such pharmacological approaches targeting both central and peripheral aspects of amyloid beta elimination, jointly involving the brain as well as the vascular and hepatobiliary systems, may have appreciable interventional potential in AD.

Furthermore, we have developed a histologically-validated MRI-DTI approach that shows that axonal white matter tracts may function as migration scaffolds of amyloid beta spread in the human brain, which has implications for the different biological subtypes of the AD syndrome. Our initial study revealed that the combination of rifampicin, cilostazol, and metformin was more synergistic than other combinations in the generalized form of AD subtypes. Subsequently, we have found the different neural regions activated by these

drugs separately in AD patients and this neuroanatomical network segmentation may provide therapeutic efficacy, of each of the three drugs in a specific neuroanatomical subtype of AD, such as the frontal, occipital and parietal variants of AD.

Our next study on therapeutic intervention for neurotropic virus infections mainly COVID19 and Japanese Encephalitis, indicates that phytochemicals and secondary metabolites have both virostatic and virucidal mechanisms to ameliorate infection of central nervous system. We developed an innovated double-hit mathematical model of these two anti-viral mechanisms, and our mathematical formulation is validated by clinical trial findings. Phytochemicals such as podophyllotoxin and quercetin have a greater binding affinity to the virus protease, suggesting their strong virucidal mode of action. Our network pharmacology approach substantiates the possible mechanism through which the phytochemicals and secondary metabolites have seminal possibilities in alleviating CNS infection. Additionally, neurally ascending viral infection can induce respiratory failure by affecting the midbrain, and phytochemicals with a satisfactory CNS permeation profile may offer a novel therapeutic avenue to counter neurotropic viral infections.

10.2 Future scope of our research

The consistentnly lower efficacy of the currently available neuroprotective drugs in clinical trials has cast doubt on the feasibility of neurorestoration approaches to treating Alzheimer's disease and neurotropic infection in humans. Our proposed novel mechanism of amyloid beta clearance in AD offers a new strategy for intervening in the disease by focusing more on peripheral amyloid elimination and the liver's pivotal role in it. Our investigated hepatomodulatory repurposed drugs transpired to be effective individually in AD and their combination therapy can target multiple pathophysiology of the multifaceted AD, hence providing the basis of a robust therapeutic intervention. Clinical trial of these

repurposed drugs (rifampicin, metformin and cilostazol) combination may be useful in preventing AD and one may actively consider undertaking such clinical trials.

Our second investigation revealed that serum cholic acid decline can be a major factor in AD, and can causatively increase brain amyloid. Our findings also indicate that this higher amyloid load in turn can causatively reduce cerebral blood flow in AD patients. Monitoring serum cholic acid levels may enable a cost-effective implementation of preventative initiatives for the general population against AD. This serum cholic acid measurement may be a preliminary screening test, and should be supported by specific confirmatory diagnostic investigations to prevent false positives. Further, exploratory clinical trials may be harnessd for the measurement of serum cholic acid and plasma amyloid beta levels for their potentiality as pre-screening tools to find those at high risk of developing Alzheimer's disease or mixed dementia.

Our third study proposed a unique approach of therapeutic intervention where we have validated that our investigated repurposed drugs could target three important affected brain regions simultaneously and provided state of the art of neuroanatomical based drug targeting in Alzheimer’s disease. This perspective could be useful while dealing with the multifacet AD syndrome which damaged several brain areas concurrently, such as the different subtypes of AD. Here also, there is scope of beta level clinical trials for exploring the clinical efficacy from a personalized neuro-anatomically based perspective.

Our final investigation identified some antibiotics and phytochemicals which could provide therapeutic efficacy in neurotropic viral infections, specifically JE and SARS-CoV2. An interesting finding was made, namely that an ascending viral infection travelled retrogradely along nerve fibres could cause respiratory failure by affecting the midbrain. As a result, preliminary clinical trials may be considered for testing of the phytochemicals,

which have a satisfactory CNS permeation profile, to offer a novel therapeutic avenue to ameliorate neurotropic viral infections that are resistant to existing treatments modalities.