DELIVERY OF PHYTOCONSTITUENTS VIA BRAIN LYMPHATICS: AN UNEXPLORED OCEAN OF OPPORTUNITIES IN TREATMENT OF BRAIN DISORDERS

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ABSTRACT:
Herbal formulations for brain health (nootropic formulations) have been used in India for millennia, and till recently, no proper exegesis could be offered to explain their effects. The very recent discovery of the existence of brain lymphatics, and their communications with systemic lymphatics, has suddenly birthed a whole new field of drug delivery to the brain. This discovery of a rich network of lymphatics in close approximation to the dural venous sinuses, a lymphatic plumbing system in effect, was found to be draining interstitial fluid and cerebro-spinal fluid (CSF), and is a landmark discovery in the field of human anatomy. Lymphatics everywhere are rich in immune cells and lipids. The high percentage of lipids in the brain composition suggests that dietary lipids can play a significant role in the structure and function of the organ. It also suggests that lipoidic bioactive molecules absorbed through the gut would majorly impact brain disorders. It is interesting to note that the vast majority of Ayurvedic preparations for brain disorders are lipid based and thus the lipoidic herbal formulations can reach the brain through lymphatic communications and exert their actions.

Keywords: Phytoconstituents, Brain Disorders, Brain Lymphatic System, Lipoid drugs, nootropic herbs, lipophilic bioactives, herbal emulsion formulations

I. INTRODUCTION

Delivery of medicaments to the brain is still considered very complicated due to presence of blood brain barrier (BBB) which prevents macromolecules from reaching the brain. The recent discovery of meningeal brain lymphatics and their communications with peripheral lymphatics, including through the nasal mucosa, has opened up vast new possibilities. The identification of this meningeal “door”, the entry and exit points for immune cells, cytokines, antigens derived from brain tissue, as also waste molecules might allow for BBB bypass, providing an interface for the immune system at large to interact with the CNS, and for lipoidic phytochemicals to access the brain.

There already exists a vast armamentarium of herbal nootropic formulations developed in ancient India. These medhyarasayans are inscribed in the Ayurvedic texts and are extolled for their efficacy. These nootropic formulations are designed for use both through the nasal route, as well as through the mouth. Though several oral dosage forms are in herbal decoction or powder forms, many others are lipid based, and herein lies their uniqueness. The brain being almost entirely composed of lipids, possesses a natural affinity for lipophilic dosage forms and thus the lipid-based herbal formulations after accessing the brain through lymphatic communications can deliver their phytoactive payload to neurons and glial cells.

Brain composition and lipophilic bioactives

The brain is composed primarily of lipids, which account for 60% of the brain mass, most of the rest being water. More than 50% of brain dry mass is membrane lipids, mainly phospholipids (O’Brien and Sampson, 1965); the phospholipids in the brain are mainly composed of omega-3 and omega-6 fatty acids (Sinclair et al., 2007). These membrane lipids play a critical role in regulation of receptor modulation and signalling. Apart from membrane lipids, the maximum amount of lipids are present in myelin sheath (80% lipids and 20% proteins). For each
protein molecule in myelin, there are 186 lipid molecules, 111 of which are polar lipids and 75 of cholesterol. The high percentage of lipids in brain composition suggests that dietary lipids can play a significant role in the structure and function of the brain. Moreover, the brain would have special affinity for lipoidic bioactives delivered by mouth, nose or via the lymphatic vasculature.

Absorption of lipoidic phytochemicals

Since lipophilic phytochemicals are absorbed from the intestinal lumen only after emulsification and micellization, the first step that takes place in the duodenum and proximal jejunum is the extraction of the compounds from herbal formulations and their incorporation into mixed micelles. The emulsification and breakdown of lipids and lipoidic formulations takes place under action of bile salts and lipase enzymes. Bioactives like Phytosterols need an emulsion vehicle to diffuse in the aqueous lumen system of the gut and cross the lipid membrane of intestinal mucosal lining cells (Epriliati et al., 2012). Even for phenolics, it requires lipid-complex called phytosome to penetrate gut lining and to enter the circulatory system. (Kidd and Head, 2005).

After absorption into the mucosal cells, the lipids and lipoidic phytochemicals like retinoids get incorporated into chylomicrons (CM) and enter the lacteals and then the mesenteric lymphatics. The mesenteric lymphatics drain into the thoracic duct which carries the lymph to the deep veins of the neck. The CM thus follow a different path from the hydrophilic molecules like amino acids and monosaccharides, which enter the portal circulation to reach the liver (see figure).

Emulsions as favoured dosage forms

Emulsions as herbal drug delivery platforms have only recently been recognized. By contrast, in Ayurveda, hundreds of emulsion formulations based on ghee or sesame oil are described, both for promoting brain health and for amelioration of brain disorders. Both of the lipid carriers employed in making Ayurvedic formulations, ghee and sesame oil have good amounts of phospholipids which act as emulsifying agents. This emulsion form of phytochemical drug delivery is now being acknowledged, since it has been recently discovered that many of the phytochemicals need to be in emulsion system to make them more bioavailable (Kang et al., 2010). Also significant is the fact that most of the bioactive phytochemicals are themselves lipophilic in nature, and require an emulsion formulation to be properly absorbed.

Traditional Indian Medicine describes many orally ingestible formulations that incorporate medhyarasayans (brain tonic herbs) which are esteemed for their efficacy. Though several dosage forms are in herbal decoction or powder forms, most of these are lipid based emulsions, and are thus remarkable for this ingenious mode of delivery. Even in the case of decoctions and herbal powders, several of these are recommended to be taken alongwith ghee. In the intensely churning milieu of the stomach, the decoction taken with ghee would surely form an emulsion.

Arrangement of brain lymphatics and landmark studies

The discovery by Aspelund in the year 2015 and Louveau in 2017 revealing a rich network of dural lymphatics suddenly demolished the neurological dogma of the brain being devoid of a lymphatic circulation, and provided a convincing explanation for the pathway whereby the lipid-based herbal formulations could access the brain. Most lymphatics from the head and neck join the thoracic duct, which also collects and carries lymph from the intestines, oesophagus and chest (see Figure). The portals for exit of the lymphatic channels from the skull, which encases the brain, are along the cranial nerves and major cerebral blood vessels. Some dural lymphatics also exit through the cribiform plate at the roof of the nose. Louveau and Aspelund showed that the sub-dural lymphatics communicate with systemic lymphatics through the submucosal nasal lymphatics as well as via the deep cervical lymph nodes (DCLN). Also, their studies have conclusively proven that the CSF alongwith macromolecules drains via the dural lymphatic vascular system into the DCLN (Louveau et al., 2015; Aspelund et al., 2015). A very recent study by Zhao and Le demonstrated elegantly that the reverse is also true, i.e., nanoparticles can be transported into the brain parenchyma from the deep cervical lymph nodes. In their study, Indocyanine green (ICG)-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles were injected subcutaneously in the mouse neck near the superficial and deep cervical lymph nodes. NIR fluorescence imaging of mouse brain tissues at multiple time points showed that nanoparticles achieved significantly high accumulation in brain parenchyma (44-fold higher than the intra-venous route) (Zhao et al., 2020). The peak concentration was achieved around 24 hrs after administration. When the same experiment
was conducted after ligating the cervical lymphatics, the concentrations achieved in the brain were considerably less, suggesting strongly the role of cerebral lymphatics in the retrograde delivery of the nanoparticles to the brain.

**Path followed by lipoidic phytoactives to reach the Brain**

Till recently, no proper justification could be offered to fully explain the effects of the herbal emulsion formulations, since the blood-brain barrier prevents macromolecules in the blood from reaching the brain. The age-old dogma of the brain being isolated and barricaded has been partially disrupted by the work of Louveau and Aspelund, and promises to be further tattered by the studies conducted by Zhou. Considering the significant study by Zhou demonstrating retrograde flow from the DCLN to the brain, and by Louveau and Aspelund demonstrating brain lymphatics, the authors propose the following hypothesis:

The lipophilic phytochemicals extracted from the herbs forming the ingredients of the Ayurvedic emulsions, after micellization in the duodenum, get absorbed by intestinal mucosal cells (enterocytes). These absorbed lipophilic bioactives are then incorporated into chylomicrons (CM) in the enterocytes, and secreted into the mesenteric lymphatics. The phytochemical laden chylomicrons bypass the first-pass metabolism in the liver and travel via thoracic duct to reach the heart, lungs and brain where the substances carried by these chylomicrons are taken up by tissues under action of CD32 and CD36. Those phytochemicals which are incorporated into very low-density lipoprotein (VLDL) after metabolism in the liver, are also secreted into the lymphatics and reach the thoracic duct. In the Thoracic Duct, these phytoactive laden lipoprotein particles travel cranially alongwith the flow of lymph, to empty into the left Subclavian Vein just before the latter joins the Superior Vena Cava to enter the heart (see figure).

Right before the thoracic duct joins the left subclavian vein, it is joined by the jugular trunk which drains the lymph from the cervical lymph nodes. Due to this communication between the thoracic duct and the cervical lymphatics, some of the lipoprotein (LP) particles in the thoracic duct can travel up the jugular trunk of cervical lymphatics to reach the sub-dural lymphatics. Even otherwise, the lattice-like inter-communications between thoracic and deep cervical lymphatics will provide alternate channels for the LP particles to travel towards the dural lymphatics surrounding the brain.

This is possible because the meningeal lymphatics, especially along the vault do not contain any valves, thus permitting bi-directional flow. Once inside the dural lymphatics, the LP particle-bound phytoconstituents can easily access the brain, since the meningeal lymphatics have no basement membrane and wide gaps are present between the endothelial cells, allowing the LP particles and carried phytochemical molecules to pass into the brain tissue. Recent work by Louveau has described how the extensive meningeal lymphatic vessel network serves as a macromolecular clearance and immune cell trafficking system in the brain. Retrograde flow of solutes as well as fluid in the peri and paravascular space in brain has been previously demonstrated by Nakada and Faghih (Nakada and Kwee, 2019; Faghih and Sharp, 2018).
Role of lipid uptake receptors in the Brain

CD36 and GPIHBP1 are lipid receptors expressed in capillary endothelial cells. Once the lipoproteins and albumin-bound bioactives reach the brain via the lymphatics, these receptors can help in uptake of the lipoidic phytoconstituents loaded onto the lipoproteins. Besides endothelial cells, CD36 is also expressed on adipocytes, microglia, monocytes and macrophages. This immuno-metabolic receptor has a high affinity for long chain fatty acids (LCFA) which it uptakes from triglycerides and albumin. Since poly unsaturated fatty acids (PUFA) are important for brain function, uptake of these by microvascular endothelial cells in the brain is important for brain health (Murphy, 2017).

GPIHBP1 strongly binds lipoprotein lipase (LPL) and serves as an important platform for the LPL-mediated processing of chylomicrons in capillaries since it binds both LPL and Chylomicron (Beigneux and Brandon 2007). It has been found to be important for the delivery of lipid nutrients to cells.

CD 32 is present on dendritic cells, microglia, neutrophils, monocytes, basophils, mast cells and platelets (Jurga 2020). Though mostly involved in internalization of immune complexes, it also has a role to play in phagocytosis of lipoproteins like LDL which are oxidized in inflammatory conditions.

All the receptors given above are involved in uptake of lipid molecules and lipoproteins. They are then also capable of taking up lipoidic phytochemicals into the brain cells on which they are expressed. The lymphatic endothelial cells on which these are expressed can take up the bioactives and transfer them to the immune and glial cells. The glial cells expressing these receptors can also bind the phytochemical loaded lipoproteins, and engulf them since the microglia behave like macrophages of the central nervous system.

II. CONCLUSION

The lipoidic bioactive molecules incorporated into chylomicrons (CM) bypass the metabolic transformation in the liver, and reach the heart and brain unchanged through the thoracic duct (which empties into subclavian vein at root of neck). Those bioactives that reach the liver carried by chylomicron remnants via normal circulation, are incorporated into VLDL and are also secreted by liver into the thoracic duct. Some of these CM, VLDL, HDL...
and LDL particles can reach the brain through the rich inter-communications between neck and thoracic lymphatics, as also through the jugular trunk, which drains the lymph from deep cervical lymph nodes into the thoracic duct. Taken together with the findings of Zhou et al demonstrating retrograde flow to brain, it will be no surprise that the lipoidic phytochemicals derived from the lipoproteins or carried by albumin will be available to the brain tissues, especially the microglia and immune cells there. It is thus clear that the bioactives within the orally administered Ayurvedic emulsion formulations can reach the brain and exert their actions for promotive and therapeutic effects.

III. ABBREVIATIONS

BBB- Blood Brain Barrier, CM- chylomicron, CNS- central nervous system, CSF- Cerebro- spinal fluid, DCLN – deep cervical lymph nodes, HDL – high density lipoproteins, LP – lipoprotein particle, LDL – low density lipoproteins, VLDL- very low density lipoproteins,

REFERENCES