ECRONICON OPEN ACCESS

EC GASTROENTEROLOGY AND DIGESTIVE SYSTEM

Mini Review

Newly Discovered Brain Lymphatics Engender Fresh Possibilities of Delivering Lipid Soluble Nootropic Phytochemicals through Lymphatic Route

Sharadendu Bali[1]* and Randhir Singh[2]

[1]*Professor of Surgery, MMIMSR, Mullana, Ambala, Haryana, India*

[2]*Professor, MM College of Pharmacy, MMDU, Mullana, Ambala*

***Corresponding Author:** Sharadendu Bali, Professor Surgery, MMIMSR, Mullana, Ambala, Haryana, India.

Received: October 04, 2021; **Published:** October 29, 2021

Abstract

Drug delivery to the brain has always been purportedly troublesome and formidable, since the Blood-Brain Barrier (BBB) is believed to be preventing access. With the recent dismantling of the age-old neurological dogma of the brain being devoid of lymphatic circulation, the scope of delivering drugs to the brain has suddenly widened considerably. There is tremendous excitement in the pharmaceutical world, as the probability of delivering drugs to the brain through the nasal lymphatic communications opens up several new prospects. But nasal delivery to brain is just a sliver of the full realm of lymphatic delivery to the central nervous system: per-oral delivery of drugs and bioactives directed to the brain is a vastly more diverse field with enormous potentialities.

In fact, brain function promoting (nootropic) herbal formulations have been traditionally used in India for thousands of years. Most of these formulations use oils or fats (lipids) as the drug carrier. This pharmacologic drug delivery mechanism (using lipids as delivery system) is now being recognized as most suitable for phytochemicals, not only because most phytochemicals are lipophilic, but also because the lipoidic molecules, after oral ingestion are transported primarily by the lymphatic system. The rich inter-communications between cerebral and peripheral lymphatics renders the transport of these lipid-carried bioactives to the brain a very real possibility.

Keywords: *Blood-Brain Barrier (BBB); Lipid Soluble Nootropic Phytochemicals; Lymphatic Route*

Brain lymphatics

Recently, the brain has been found to have a well-formed lymphatic drainage system to clear macromolecules and debris. This discovery was made in rodents only as late as 2015 and confirmed in humans in 2017. These expositions made by Aspelund [1] and Louveau [2] demonstrated the presence of an abundant plexus of lymphatic vessels underlying the large venous sinuses in the vault of the brain. The cerebral lymphatics were also found to be communicating with the systemic lymphatic system through the submucosa of the nose and the deep neck nodes. In the main, the brain lymphatics exit the cranium through the various foramina of the cranial nerves and also along the large blood vessels. The cribriform plate allows for the passage of numerous filaments of the olfactory nerve, as also the accompanying plentiful lymphatics.

Lipid composition of human brain

The brain has the second highest lipid content in the human body, the maximum amount being obviously in the adipose tissue. Brain lipids constitute more than 50% of the dry weight of the brain, most of which is in the form of phospholipids amalgamated into cell

membranes [3]. The fatty acids (FA) present in brain composition are mainly long-chain polyunsaturated FAs, primarily arachidonic acid, docosahexaenoic acid and eicosapentaenoic acid [4]. Synthesis and breakdown of the FAs in the brain takes place on a daily basis, with around 8% of the PUFAs being replaced by FAs obtained from the blood circulation. This high turnover of brain lipids suggests that dietary lipids would have a significant role to play in brain lipid composition and function of cells constituting brain tissue, primarily neurons, supporting glia and resident immune cells. Lipoidic phytoconstituents of lipid-based nootropic formulations too, by the same logic, would be avidly taken up by brain tissue and become available to wield their pharmacologic actions.

Drug delivery to brain

Ferrying drug molecules to the brain tissue is still reckoned as challenging due to the obstacle provided by the blood brain barrier (BBB) which prevents solutes in the blood from crossing over into the brain. So, therapeutically active molecules that can treat brain maladies cannot reach the brain cells easily when blood is the transport media. Though exosomes have been used, these have achieved only limited success. Using pro-drugs, disguised with lipophilic molecules, has also been tried to allow the active molecule to sneak through the BBB. Peptide masking by cholesteryl molecule has also been tried.

The latter methods utilizing lipid molecules were partly successful because the cell membranes of the BBB are made up of lipids. Also, because of the high percentage of lipids in brain tissue, lipoidic bioactive molecules have an enhanced propensity for uptake by brain. Since the vast majority of the Ayurvedic preparations for brain health and neurological disorders are lipid-based formulations, they are suitable for delivery to the central nervous system. The discovery of brain lymphatics has now expounded a convincing route for these lipoidic formulations to reach the brain and exert beneficial effects.

Absorption and transport of lipophilic phytochemicals

After oral ingestion, lipid globules are broken down into smaller particles in the duodenum and proximal jejunum by the processes of emulsification and micellization. This breakdown of fats into smaller molecules takes place under action of bile salts and pancreatic lipase enzyme. The efficiency of this step is only partial, and the fraction of fat-soluble phytochemicals that is thus made potentially available for absorption is referred to as the bio accessible fraction [5]. The FAs and lipophilic bioactive compounds are then able to be absorbed by the intestinal mucosal cells. Essentially, an emulsion vehicle is required for the lipophilic phytochemicals to diffuse in the aqueous environment of the gut lumen, and to penetrate the mucosal cells lining the inside of the gut [6]. Even for hydrophilic bioactives like phenolics, a lipid vesicle-complex called phytosome is required to cross the intestinal mucosal lining and to enter the blood circulation [7].

Traditional Indian Medicine describes a large number of formulations for promoting brain health, and for amelioration of brain disorders. The promotive nootropic formulations, called Medhya Rasayans, are oral formulations having a salutary effect on the functioning of the brain, increasing memory, cognition and concentration. The most effective formulations for this purpose are the ghritas, which are preparations processed in ghee (clarified butter). Since the ghrita formulations entail cooking herbal pastes in herbal decoctions and ghee, the final product is an emulsion.

The importance of emulsions as herbal drug delivery platforms has already been described above. What is important to note is that the use of emulsions to increase the bioavailability of phytochemicals has only been recognized over the last decade [8].

In Ayurveda, hundreds of emulsions systems are described. These are either ghritas, or awalehas which are confections cooked in ghee or sesame oil. Some herbal formulations in powdered form are prescribed to be taken along with ghee, in which case also emulsion will be created in the aqueous environs of the gut. After absorption of the FAs, glycerides, cholesterol and lipoidic phytochemicals into small intestinal mucosal cells as described above, the former, in association with apoproteins (to make them hydrophilic) form lipoprotein particles called chylomicrons and are secreted into the lacteals in the intestinal wall. The small intestinal lacteals all drain into the mesenteric

lymphatics, from where the lymph collects in the cisterna chyli to travel cranially through the thoracic duct. This duct, just after being joined by the jugular lymphatic trunk in the neck, empties into the left subclavian vein.

The hydrophilic molecules like amino acids, sugars, water soluble vitamins and alcohols, on the other hand, enter the portal vein which supplies blood to the liver (see figure 1). Hence, after a meal, while the water soluble food substances reach the liver, the lipids and lipophilic substances are carried by the lymphatics to directly reach the right heart. The lipoidic phytoconstituents are thus spared the metabolic transformation which awaits any molecule reaching the liver. These virgin bioactives, then, can exert their action on the heart, lungs and brain before being metabolized and transformed in their subsequent passage through the liver. Those lipoproteins that are not up taken by the cardio-pulmonary tissues, enter the left heart from the lungs and join the general blood circulation through the aorta. Through the blood circulation, upon reaching the liver, these lipoproteins and bound phytochemicals are then taken up by liver cells, broken down and re-secreted into blood as VLDL particles. Some phytochemicals are also carried in blood and lymph bound to albumin.

Lymphatic route for lipoidic bioactives to reach the brain

The discovery by Aspelund in the year 2015 and Louveau in 2017 of a lattice-like profusion of lymphatic vessels in the sub-dural space, upended the centuries old anatomical doctrine of the brain not being endowed with a lymphatic circulation. It also illuminated the probable pathways whereby the phytoconstituents of traditional Indian medicine formulations can be transported into the brain, allaying the scepticism skepticism surrounding the use of these formulations.

In brief, the lymph from the from the left side of the head and neck drains into the jugular trunk in the neck, which subsequently joins the thoracic duct. Lymph from the right side of head joins the deep veins of neck directly. The thoracic duct, which collects and carries lymph from the whole abdomen and chest, including the dietary lipids absorbed through the gut, drains into the left subclavian vein soon after the duct is joined by the jugular trunk. Classical studies by Louveau and Aspelund and several other workers have already demonstrated that the CSF and immune cells from the brain drain into the deep cervical lymph nodes (DCLN). It is also well known that the lymph from the DCLN drains via the jugular trunk into the thoracic duct.

A recent elegant study in 2020 by Zhao and Le [9] has shown that the converse is also true, namely, that macromolecules can be conveyed to the brain tissue from the cervical lymph nodes; in other words, retrograde flow from DCLN to the brain exists. In their experiment, Zhao and colleagues loaded Nanoparticles of PLGA (poly lactic-co-glycolic acid) with Indocyanine green (ICG). These particles were injected subcutaneously in the mouse neck near the superficial and deep cervical lymph nodes. The animals were then sacrificed at multiple time points (18 - 48 hrs), and imaging of mouse brain tissues by NIR fluorescence was carried out. The infra-red fluorescence imaging demonstrated that the ICG-loaded PLGA nanoparticles accumulated in significant amounts in brain parenchyma (44 times higher concentration than the nanoparticles injected intra-venously). This effect was abrogated if the deep cervical lymphatics were ligated, proving that the nanoparticles reached the brain through the cervical lymphatic communications.

Taking into account the study by Zhou showing reverse flow, and by other workers proving the existence of brain lymphatics, the authors advance the hypothesis stated below.

The lipophilic phytochemicals present in the herbs forming the ingredients of the Ayurvedic lipid-based drug delivery systems (LBDDS), get incorporated into chylomicrons in the intestinal cells (enterocytes). These chylomicrons are absorbed by lacteals and reach the heart directly. Having by-passed the metabolic degradation and transformation in the liver, the phytochemical-loaded chylomicrons travel cranially unchanged through the thoracic duct (See figure 1). Those phytochemicals incorporated into very low-density lipoprotein (VLDL) in the liver and secreted into the lymphatics also reach the thoracic duct. The latter is joined by the jugular trunk just before its termination. Since there is a valve at the mouth of the thoracic duct, resulting in relative stasis of lymph flow in the vicinity of the confluence with the jugular trunk, some of the lipoprotein particles (chylomicrons, VLDL and HDL) can head cranially to reach the DCLNs. These

phytochemical-loaded lipoproteins can then travel up the lymphatic channels of the neck to reach the sub-dural lymphatics, since the meningeal lymphatics along the vault do not contain any valves, thus permitting bi-directional flow.

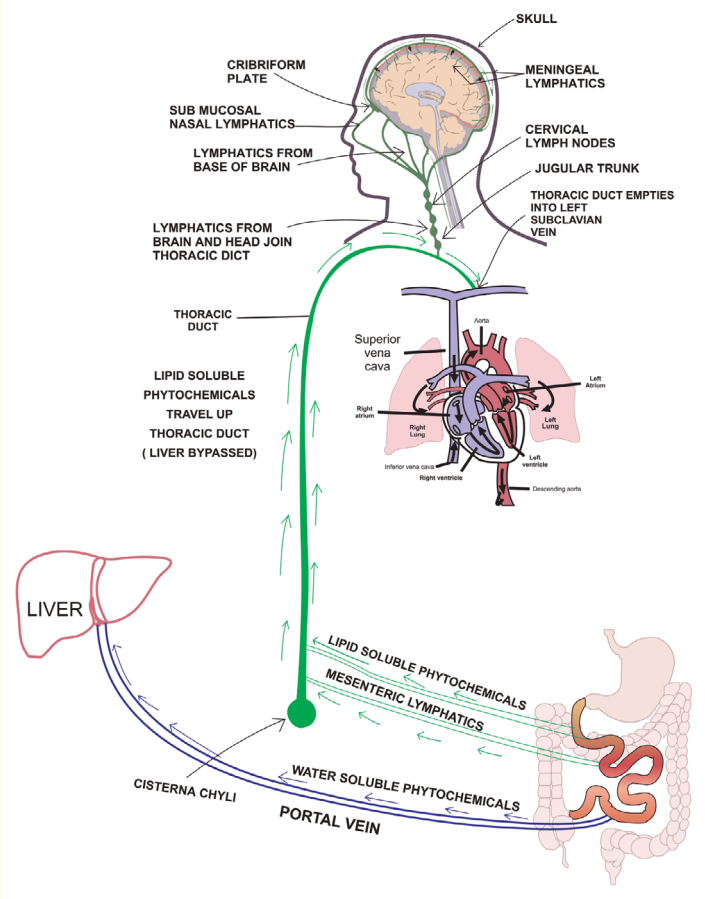

Figure 1: *Figure showing route of transport of lipid soluble phytochemicals, bound to lipoproteins or albumin, from intestines to heart and brain through thoracic duct.*

Retrograde flow of macromolecules as well as CSF in the peri and paravascular space (glymphatic system) in brain has also previously been demonstrated by Nakada [10] and Faghih [11,12].

Once in the sub-dural lymphatics, the bioactives can be taken up by endothelial cells and immune cells. Some lipoprotein particles can also directly reach the brain tissue, because the basement membrane is absent in the meningeal lymphatics, and endothelial cells are spaced out, leaving broad gaps in between them. In the brain, the lipoidic bioactives carried by the lipoproteins can be taken up into brain cells (neurons and microglia), as well as immune cells resident in cerebral lymphatics, under action of CD 32 and CD 36 receptors.

Conclusion

Though the phytochemicals carried by lipoproteins in the blood cannot easily access the brain due to the BBB, the bioactives bound to the lipoproteins (chylomicrons, VLDL and HDL) in the lymphatics can reach the cranial cavity through the communications described

above. Inside the lymphatics of the cranial cavity, the bioactives can be either taken up by resident immune cells or by endothelial cells. The lipoidic phytochemicals carried by lipoproteins and other protein carriers that reach the brain through the lymphatics, can be imbibed by all the brain cells, including neurons and glia, but especially the macrophagic microglia. Lipid based drug delivery can thus prove to be a potential game-changer in the treatment of neurological disorders.

Bibliography

1. Aspelund A., *et al*. "A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules". *Journal of Experimental Medicine* 212.7 (2015): 991-999.
2. Louveau A., *et al*. "Structural and functional features of central nervous system lymphatic vessels". *Nature* 523.7560 (2015): 337-341.
3. O'Brien JS and Sampson EL. "Lipid composition of the normal human brain: gray matter, white matter, and myelin". *Journal of Lipid Research* 6.4 (1965): 537-544.
4. Sinclair A., *et al*. "Omega 3 fatty acids and the brain: review of studies in depression". *Asia Pacific Journal of Clinical Nutrition* 16.S1 (2007): SS391-S397.
5. Borel P and Desmarchelier C. "Bioavailability of fat-soluble vitamins and phytochemicals in humans: effects of genetic variation". *Annual Review of Nutrition* 38 (2018): 69-96.
6. Epriliati I., *et al*. "Bioavailability of Phytochemicals, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health". Dr Venketeshwer Rao (Edition.) (2012).
7. Kidd P and Head K. "A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex (Siliphos)". *Alternative Medicine Review* 10.3 (2005): 193-202.
8. Kang J., *et al*. "Non-isoflavone phytochemicals in soy and their health effects". *Journal of Agricultural and Food Chemistry* 58.14 (2010): 8119-8133.
9. Zhao P., *et al*. "Therapeutic delivery to the brain via the lymphatic vasculature". *Nano Letters* 20.7 (2020): 5415-5420.
10. Nakada T and Kwee IL. "Fluid dynamics inside the brain barrier: current concept of interstitial flow, glymphatic flow, and cerebrospinal fluid circulation in the brain". *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry* 25.2 (2019): 155-166.
11. Faghih MM and Sharp MK. "Is bulk flow plausible in perivascular, paravascular and paravenous channels?" *Fluids and Barriers of the CNS* 15.1 (2018): 1-10.

Volume 8 Issue 11 November 2021