CHAPTER 9
General discussion, conclusions and perspectives
The understanding of the molecular mechanisms of AD and stroke together with the urgency to find new therapies are closely linked with the high impact that this research could have in society. Neurodegenerative disease is a general term for a range of conditions which mainly affect neurons in the human brain. The prevalence of neurodegenerative disorders is increasing, owing in part to extensions in lifespan [2]. Nowadays, there is no cure for any of these diseases, so our goal in this thesis was to shed more light on the molecular mechanisms of these diseases by focusing on a lipidomic approach and evaluate a potential protection of a naturally occurring terpene alcohol, namely linalool, in some of the most common neurodegenerative conditions such as AD and ischemia.

Since ancient times, humanity has used medicinal plant preparations for the treatment of its diseases, and as research has evolved, new technologies have enabled more studies on natural compounds which derive from plants [2]. The health benefits reported by other authors [3] and preliminary studies in our group were the reason to choose different approaches to evaluate the protection of the naturally occurring monoterpene linalool. We described in two in vitro models for oxidative stress associated to neurodegenerative conditions (HT-22 cell line & primary neurons and astrocytes), one ex-vivo model for excitotoxicity associated to neurodegenerative conditions (OHSC) and two in vivo models for AD and ischemia (3xTg-AD mice & 2-VO in rats) the protection of the monoterpene linalool (Figure 1).

Linalool is susceptible to chemical modifications (oxidation, glycosylation, esterification, and methylation) due to the two double bonds and the hydroxyl group (OH) in its structure [4,5]. Linalool exhibits chiral properties due to the OH group in the third carbon (C3), and it has two enantiomers and the racemic (rac) form. The pharmacological differences between linalool enantiomers have been shown on physiological parameters on human stress reactions assessing endocrine and autonomous nervous system [6] and in sedative effect in humans [7]. The chiral influence of other optically active monoterpenes on behavior experimental tests in mice also was confirmed [8]. Interestingly, Sousa et al., 2010 showed that both enantiomers and rac-linalool have similar anticonvulsant activity, but differ in their potencies where rac form was the most potent [9].

Linalool is neither phototoxic [10] nor genotoxic [10,11]. The evaluation of linalool in vitro in bacterial test systems (Ames assay) and in vivo in mammalian test systems (micronucleus assay) showed no evidence of mutagenic or genotoxicity [12]. Nevertheless, the toxicity was evaluated in Sprague–Dawley rats with the maternal NOAEL (No observed adverse effect level) and the developmental NOAEL were 500 mg/kg bw/day and 1000 mg/kg bw/day, respectively [12]. Besides, after oral exposure in rats, NOAEL value was 500 mg/kg bw/day and the margin of safety was major to 5000 [12]. These concentrations are much higher compared with 25 mg/kg that we and other colleagues have used in models of AD, ischemia or other disease models [14–17].

Figure 1. Protection by linalool in different models of neurodegenerative diseases. Green arrows represent the linalool effect on the therapeutic targets A. Primary and HT-22 cells protection by linalool: neurons and astrocytes in culture were tested with excitotoxicity by glutamate and linalool we showed linalool recovered the cell viability and levels of ATP; decrease the actin depolymerization and cell death by linalool; In HT-22 cells linalool reduced ROS, calcium mitochondrial, recovered mitochondrial membrane potential and increased maximal respiration in mitochondria. B. We observed in OHSC that linalool reduced cell death mainly in CA1 from the hippocampus and COX-2 expression after NMDA toxicity. C. Oral administration of the linalool ameliorated β-amyloidosis, tauopathy, microgliosis and astrogliosis through regulation of proinflammatory markers as COX-2, NOS2, IL-1B, P38MAPK in an aged triple transgenic AD model mouse. D. Linalool reduced astrogliosis and microgliosis in the hippocampus modulating COX-2 protein of rats subjected to 2 vessels occlusion (global cerebral ischemia); we found linalool was able to maintain the homeostasis of lipids such as LPC 22:6, LPE 22:6, PC 36:1 and PC 42:1 in hippocampus and PI 36:2 in serum. E-G. Linalool reduced cognitive and emotional deficits in 3xTg-AD and sensorimotor and memory deficits in ischemic rats.
Experimental studies on rats, performed with 14 C-labelled linalool (500 mg/kg bw), indicated that it is quickly absorbed from the intestinal tract after oral or gavage administration. After absorption, most of the linalool is quickly metabolized in the liver to polar compounds that are mainly excreted in the urine as free form or conjugates. As an example, Aprotosoaie et al, 2014 detected 8-Hydroxy- and 8-carboxy-linalool as significant metabolites following 20 days administration of 800 mg linalool/kg bw/day [18] or dihydro-, tetrahydro-linalool identified in rodent urine. Besides, a high proportion of oral linalool follows intermediary metabolism pathways and is eliminated in exhaled air as CO₂ [22].

We demonstrated the protection of linalool in vitro in a model of 1) excitotoxicity induced by glutamate in neurons and 2) of oxtosytis in neuronal HT-22 cells. HT-22 cells lack NMDAR; therefore glutamate stimulation initiates a distinct pathway of cell death independent of NMDA-related increased neuronal excitability. Our data are supported by previous in vitro studies in which PC12 were challenged by glucose/serum deprivation (GSD) conditions, and linalool was able to prevent the cell death following 8 hours of GSD [19]. In parallel, Park et al., described neuroprotective effects of linalool against oxygen glucose deprivation/reoxygenation (OGD/R) that induced cortical neuronal injury. This study showed that linalool significantly reduced intracellular oxidative stress, scavenged peroxo radicals, and decreased the activities of SOD (superoxide dismutase) and catalase [20]. In vitro screening of linalool’s antioxidant potency depicted moderate superoxide scavenging and negligible H₂O₂ scavenging activity [21], in contrast to the protective effects against H₂O₂ induced oxidative stress in brain tissue [22].

Mitochondria play an essential role in cellular metabolism, where oxygen consumption through the electron transport system (ETS) is tightly coupled to ATP production and regulate metabolic demands. Mitochondrial respiration take part in key processes such as oxidative phosphorylation, ion gradients, membrane potential, ROS generation and heat dissipation [23,24]. We demonstrated that linalool in vitro restored intracellular ATP levels in a glutamate excitotoxicity model suggesting that mitochondria play an essential role in the linalool-mediated protection. In consequence, we conducted high-resolution respirometry experiments to evaluate mitochondrial metabolic functions. We observed an increase in maximal oxygen consumption in response to linalool. Maximal oxygen consumption rate attained by adding the uncoupler FCCP mimics a physiological “energy demand” by stimulating the respiratory chain to operate at maximum capacity, which causes rapid oxidation of substrates (sugars, fats, amino acids) to meet this metabolic challenge [25]. Our data indicate that increased maximal respiration and spare capacity in response to linalool is associated with increased metabolic fitness allowing the cell to cope better with stress.

On the other hand, linalool was able to recover the homeostasis of phospholipids such as PC in hippocampus and PI in serum in rats one-month postischemia. It has been described that linalool reduced cellular lipid accumulation regulating PPARα-responsive genes and significantly induced FA oxidation, and its effects were markedly attenuated by silencing PPARα expression. In mice, the oral administration of linalool for three weeks reduced plasma TG concentrations in Western-diet-fed C57BL/6J mice [26]. Linalool reduces the expression of 3-hydroxy-3-methylglutaryl CoA reductase via sterol regulatory element binding protein-2 and ubiquitin-dependent mechanisms [27] and inhibits proliferation and cholesterogenesis in liver-derived (HepG2) and extrahepatic (A549) cell lines [28]. Besides, linalool could inhibit intracellular lipid in 3T3-L1 adipocytes, indicating that S-(+)linalool might play an essential role in controlling body weight [29].

Interestingly, our data showed that linalool-treated 3XTg-AD mice exhibited an increased frequency of entry into the open arms, increased head-dipping, reduced grooming and rearing frequencies compared with those of vehicle-treated 3XTg-AD mice. Our data confirm the results of previous studies reporting that inhaled linalool has anxiolytic properties, increased social interaction, and decreased aggressive behavior [10,11]. Chen et al., 2015 described that linalool 500mg/kg significantly increase the time that mice spent in the open central area detected by the open field test and also the time they spent in the open arms of the EPM. This concentration did not render any side effects on motor activity, indicating excellent anxiolytic responses [30]. However, other study did not report any difference in the number of entries to the open arms using 125 mg/kg linalool [31]. In our study, we observed significant improvements in the neurological score, motor function and cognitive function by linalool 25 mg/kg given orally in 2VO model of cerebral ischemia. In accordance with the data from 3XTg:AD, linalool treatment effectively decreased the escape latencies in the learning, retention and re-learning tests compared with control groups in both models. Recently, Xu et al. corroborated this effect in another model of AD, where 100 mg/kg linalool reduced the escape latencies, increased the escape rate, and increased the time spent exploring the platform location in MWM test [32].

Reduction of COX-2, astrogliosis and microgliosis were common processes in our studied models of AD and stroke. Therefore, our data is indicative of a potential anti-inflammatory effect of linalool in conditions of stress. Inflammatory reactions, caused by diseases such as diabetes and cardiovascular disease, can be alleviated by linalool, which interacts with proteins such as COX-2, NF-kB, and nuclear factor erythroid 2-related factor 2 (Nrf-2) [33]. Linalool, mainly (+)-linalool, may also interact with nitric oxide synthase (NOS) enzyme, inhibiting the production of nitric oxide (NO) without reducing the enzyme synthesis [34]. Moreover, Li et al. suggested that linalool inhibited LPS-induced inflammation in BV2 microglia cells, both via the NF-κB pathway and also by the activation of Nrf2/ heme oxygenase-1 (Nrf2/HO-1) signaling pathway [35].

We observed microglia activation in AD and stroke animal models, in concordance with morphological alterations reported in postmortem AD and stroke brain tissue [36–38] suggesting that innate neuroinflammatory processes play a role in the progression of
neurodegenerative diseases. Recent findings reported a molecular diversity of murine microglia on a temporal and spatial axis during ontogeny, homeostasis, adulthood, and aging [41–43]. iPSC technology offers the opportunity of culturing patient derived cells and in this way this technology represents a valuable tool for studying disease etiologies at molecular and cellular levels. Based on these observations, we have decided to generate microglia from healthy control subjects by using a modified protocol of Douvaras et al., 2017 [44]. Protocols to generate microglia are fairly recent, just few years ago, Muffat et al, 2016 [45] described the first protocol to produce iPSC-derived microglial-like cells, and nowadays there are around 10 protocols described in literature.

These reported protocols share similar patterns in the microglial differentiation steps, with one common denominator at the beginning of the protocol being represented by BMP4 and at the end during the maturation phase IL-34, MCSF and GM-CSF. BMP4 regulates proliferation and differentiation, cell-fate determination, inhibits neurogenesis and induces neural stem cell (NSCs) glial differentiation in the adult CNS [46,47]. During differentiation, these protocols are using a variety of growth factors and interleukins such as IL-3, IL-6, TPO, VEGF, and SCF. During the last steps of differentiation, IL-34 is added together with GM-CSF to promote the maturation of microglia. These substances are also required for the differentiation, proliferation and survival of monocytes, indicating the similarities between monocyte and microglia functionality [45,46].

The efficiency in the generation of microglia progenitors showed a marked difference between EHS and iPSC lines, 23% compared to 87%, respectively. Our data are in concordance with Douvaras et al., 2017 [48] where they obtained a 40% to 60% efficiency in ESC and 45-95% using iPSC. Likewise, it has been demonstrated that generation of microglia from iPSC derived from individuals with varying disease status, age, and sex, and the reprogramming strategies highly influence the variability of yield per iPSC lines [49,51].

Our review presents an overview of the role of microglia in human neurodegenerative diseases, and different protocols that can be employed for generation of microglia from iPSC. We discuss the variability between growth factors, medium, time scale for the production of microglia that affect the yield, scalability and purity of these cells. We demonstrate the purity of microglia cells by immunostaining with markers IBA-1 and TMEM119, similarly, other groups used these markers or CD11B, CD11C, P2RY12, CD45 [45,50-52-54]. TMEM 119 (Transmembrane protein 119) has been validated as a unique marker to differentiate microglia from related cell types as macrophages or monocytes [55].

In the same way, isolation by FACs is based on different antibodies according to the microglia progenitors. Abud et al.,2017 reported the isolation of hematopoietic progenitors after sorting with CD43 [56] and Douvaras et al.,2018 described that microglia progenitors were isolated with CD14 and CX3CR1 [46]. However, functional assays are reproducible with both protocols and they reported high purity, while transcriptomic analysis in almost all the protocols showed a unique signature for microglia with genes such as Ferls, C1qa, P2ry12, Pros1, Mertk and Gas6 [54]. Whole-transcriptome principal component analysis (PCA) highlights the differentiation trajectory of monocytes, macrophages and dendritic cells compared to microglia. In addition, the comparison between generated microglia and its potential resembling with neonatal microglia or adult microglia form patients was shown [50,57]. Nevertheless, Garcia et al, 2018, point out similarities between gene expression in iPSC macrophages and iPSC microglia and therefore refer to them as iPSC-microglial-like cells (iPSC-MGLCs) [58].

In our studies, functional assays demonstrated the maturity of human differentiated microglia, as showed by their response to a variety of stimuli and detected by xCELLigence real-time impedance measurements and phagocytosis assays. Nevertheless, ongoing experiments are realized to confirm the unique signature of microglia. Discovering the pertinent immune components may lead to the development of new therapeutic targets to decelerate or even cease neurodegenerative diseases.

Lipid profile in neurodegenerative diseases

The field of lipidomics has recently converged into the neuroscience field trying to elucidate the role of lipids, to characterize their biologic role, and their relationships concerning the expression of proteins involved in lipid metabolism and function in aging and neurodegenerative diseases [59]. The composition of brain lipids in the cerebral cortex is altered with age and brain injuries [60]. In cerebral ischemia and AD processes, important changes have been determined in the composition of fatty acids, cholesterol, phospholipids, and sphingolipids. These alterations can produce other intermediates that in turn can change their overall lipid homeostasis. These processes highly depend on the time elapsed post-ischemia, stages of AD and the brain region, where the composition of lipids regulate the molecular pathways of cell death.

We evaluated the potential alteration of the hippocampal and peripheral phospholipid profile in long-term postischemia associated with cognitive impairment in rats. The main changes on phospholipids (PLs) were associated with hippocampal dysfunction, such as the decrease in phosphatidylcholine (PC 32:0, PC 34:2) and the increases in PLs dysfunction in membrane structure and signaling. In particular, lysophosphatidylethanolamine (LPE 18:1) and phosphatidylserine (PS 38:4), in the hippocampus were associated with these effects. Complementarily, PC 34:2, and ether-PC 34:1 decreased, while Lyso-PC 20:4 and phosphatidylinositol (PI) 36:2, as neurovascular state sensors, increased in the serum, as we can observe in Figure 2. Interactions between phospholipids and other lipid mediators
are involved in maintaining lipid asymmetry, a dynamic process, which they are required for maintaining neural membrane functions such as neuroplasticity and vesicular transport [61]; regulation of these functions after ischemia is fundamental to understand the pathophysiology of CVD.

Similarly, we showed differential lipid patterns between pure vascular dementia as CADASIL, sporadic Alzheimer disease (SAD) and control patients. The lipid patterns were investigated in gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). PLS-DA analysis discriminated species with the higher sMC index, such as PS 44:7 (18:1/22:6)/LPE 18:2 in GM; PE 32:2 (16:1/16:1)/PC 44:6 (18:0/22:6) in WM, and ePE 38:2 (18:0/20:2)/ePC 34:3 (14:0/20:3) in CSF. These findings were supported by high correlation profiles of PLs between both pathologies as indicated in the heat maps. Interestingly, common sub-phospholipidic species discrimination was obtained in both types of dementia in respect to the control, such as, PS 44:7/LPC 22:5 in GM, highlighting a transversal phenomenon that impact the brain parenchyma in a similar manner. Moreover, a comparable immunoreactivity pattern supported these results by neural (astrogliosis, microgliosis, dendrite loss) and vessel population (retraction and extravasation) markers in both types of dementia.

Phospholipids are biologically relevant molecules, which form cell membranes and are involved in the behavior of membrane proteins, receptors, enzymes, and ion channels. They are involved in maintaining lipid asymmetry and vesicular transport [62]. Increased activity of phospholipases, increased anabolism of lysophospholipids, peroxisomal dysfunction, imbalances in the levels of saturated/unsaturated fatty acids and oxidative stress are associated with neurodegenerative diseases and are indicative of the progress of the pathology [63].

Phosphatidylcholine (PC) is the most abundant glycerophospholipid in the membrane and plays a crucial role in cellular signaling [64]. We observed an imbalance in the production of PC and LPC one-month post-ischemia while some species of PC were reduced and some species of LPC were increased. However, in CADASIL and SAD tissue we observed both forms of PC 42:4 and 44:6 were increased. Our results in ischemia are in concordance with previous studies where PC was reduced after 24 hours following the injury [65] and also in neurodegenerative diseases. In particular, a reduction of PC and PE levels was detected in the cerebral cortex of patients with AD, which is potentially linked to the roles of PLA, PLD in BA activation [66,67]. In our study, we observed an increase of PC in SAD, while in a different study alterations in this PL in the frontal cortex, parietal, and temporal region of AD patients were not observed [68]. Based on these results, the PC distribution patterns depend on the fatty acid composition, which reflects the heterogeneous membrane lipid compositions in distinct cell types. In addition, PC species are composed of saturated palmitic (16:0) and stearic acid (18:0), monounsaturated oleic acid (18:1) and polyunsaturated linoleic acid (18:2). This composition of membrane lipids determines their fluidity, curvature, permeability and signaling pathways that can mediate proinflammatory cytokines release, and worsen the cellular homeostasis in neurological diseases. Our results confirmed the imbalance in PLs and LPLs after global cerebral ischemia, CADASIL and SAD.

Phosphatidylinositol (PI) was present in low percentages in the serum of ischemic rats. However, we observed a remarkable increase that could be used as a predictive marker in the time-course after cerebral ischemia. PI is characterized by the phosphorylation of the inositol head group of phosphoinositol, with a rapid and reversible phosphorylation rate, which critically participates in signal cascades, cytoskeletal remodeling, and intracellular membrane trafficking [69,70]. Similar studies showed stimulation of 3Hinositol monophosphate formation via excitatory amino acids signaling that is greatly enhanced in hippocampal slices 24 h or 7 days after reperfusion in a four vessels occlusion–ischemia model [71]. Furthermore, PI increased in response to calcium stimuli in the brain [71], while calcium imbalance was associated with mitochondrial dysfunction [71], vascular alterations and has been recognized to promote a toxic environment in neurological diseases [72].

Phosphatidylserine (PS) plays a central role in the brain cell membrane fluidity, transmission of brain cell activity and neural information [75]. We observed PS 42:7 was the main

Figure 2. Regulation of phospholipids after one-month post-ischemia. Phospholipids were evaluated after one month of 2 vessel occlusion model in rats (multifocal injuries) in the hippocampus and serum. We detected a reduction of PC 32:0, PC 34:2 and increase of LPE 18:1 and PS 38:4 in the hippocampus. Meanwhile, in serum we observed an increase of PI 36:2 and LPC 20:4 and reduction of PC 34:2, and ePC 34:1. Besides we observed rats with ischemia has cognitive deficits and alterations in myelin, cerebral parenchyma, and astrogliosis. LPC, Lysophosphatidylcholine; PI, phosphatidylcholine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; ePC, ether phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine.
we observed an increase of ether-PC (ePC) in the CSF of CADASIL and SAD groups. Ether lipids represent one of the major structural component of cell membranes; their incorporation in PLs alters the membrane physical properties and affects membrane dynamics. This property is particularly important in higher order membrane structures such as those found in myelin, evidenced by its enrichment in plasmalogens [80].

PLs are multifunctional molecules, are the major constituents of membranes and are responsible for the membrane bilayer. Their physiological roles have been challenging to be identified and are likely to vary in different tissues, metabolic processes, and developmental stages. Our findings are potentially useful for improving prediction and intervention after cerebral ischemia, SAD and CADASIL and form the basis for a future understanding of PL dysfunction in neurological pathologies and will help to propose new biomarkers and therapeutic targets.

Main Conclusions

Oral administration of linalool at an advanced stage of AD in 3xTg-AD model mice reversed the histopathological hallmarks of AD (amyloidosis, tauopathy, astrogliosis, and microgliosis) and restored cognitive and emotional functions evaluated by analysis of Morris water and elevated plus maze behavioural tests, respectively.

- The treatment with linalool prevented glutamate-induced cell death of neurons and astrocytes, retraction of neural processes, actin cytoskeleton depolymerization and recovering ATP levels following glutamate challenge.
- Post-ischemic neurological, motor and cognitive impairments were prevented by the oral linalool treatment. Linalool reversed the phospholipid alterations, reduced astrogliosis and COX-2 expression in the hippocampus.
- Administration of linalool to HT-22 cells challenged by toxic concentrations of glutamate, reduced cell death, mitochondrial ROS, lipid peroxidation, calcium levels, and preserved mitochondrial membrane potential.
- Linalool increases maximal respiration and spare capacity in HT-22 cells contributing to its neuroprotective capacity.
- Linalool reduced NMDA-induced damage in the hippocampus and the increase of the gene expression of COX-2 in organotypic hippocampal slices.
- Based on our findings, we propose that linalool may be a potential candidate for further preclinical studies and future translational studies on neurodegenerative diseases
- Phospholipids associated with neurotransmission, such as phosphatidylcholine (PC 32:0, PC 34:2, PC 36:3, PC 36:4, and PC 42:1) were reduced. In addition, increases in PLs involved in the membrane structure and signaling, such as lysophosphatidylethanolamine (LPE 18:1, 20:3, and 22:6) and phosphatidylserine (PS 38:4, 36:2, and 40:4), in the

Lyso phospholipids (LPLs) are proinflammatory phospholipids that are synthesized in the brain through the action of phospholipase 1 (PLA₁) and PLA₂ on PLs such as PC and PE, which are metabolized by lysophospholipases and acyltransferases. LPLs, such as LPC, not only directly interact with ion channels and neurotransmitter receptors but also indirectly modulate their activity and neural membrane fluidity [79]. LPC 20:4 and LPE 18:1 were increased in the ischemic group compared to the other groups. LPLs such as LPC and LPE alter the membrane permeability and disturb the osmotic equilibrium. On the other hand, discriminant PL in GM where in CADASIL and SAD groups the levels of PL were reduced compared to the controls. While, Wood et al., 2015 reported that the levels of PS were unaltered in the CSF, GM or WM [76], other groups reported that PS levels gradually decrease with increasing of age, affecting memory and cognitive ability. In AD cases this reduction is higher compared with control subjects [77]. Besides, we observed PS 42:7 (18:1/22:6) was composed by oleic acid and DHA was altered in dementia groups, correlating with a lower plasma concentration. In addition, other fatty acids have been associated with cognitive decline in both healthy elderly people as well as patients with AD [76].
The question of how linalool regulates the mitochondrial function and phospholipids remains to be solved. Therefore, future study involving genetic modification of mitochondria respiratory complexes and phospholipids such as PC and PI would give us insights to better understand the mechanism of action of this terpene.

Assessment of bioavailability, determination of pharmacokinetic characteristics and use of pharmacokinetic/pharmacodynamic modeling can aid in more rational use of linalool. This will allow to propose this molecule to begin phase 0 designed by the FDA on Exploratory Investigational New Drug (IND) Studies. Phase 0 trials that include the administration of single subtherapeutic doses of the study drug to a small number of subjects (10 to 15) to gather preliminary data on the agent’s pharmacokinetics (what the body does to the drugs). In this way we would be able to validate the therapeutic potential of linalool and allow in a future its pharmacological application in neurodegenerative diseases.

We showed a phospholipid profile in different diseases as SAD, CADASIL and stroke. A metabolomic profile including other lipids involved in brain damage, such as sphingolipids, gangliosides and cardiolipin, could help to understand the pathology and progression of these diseases.

We successfully generated functional human microglia from iPSC that could be used to interrogate emerging questions associated to inflammatory processes in neurodegenerative diseases.

Our findings demonstrated that administration of linalool reduce the main hallmarks in AD and in ischemic stroke, effects being associated with the improvement in motor, memory and cognitive skills. Although, more studies are necessary to properly understand its mechanism of action, we have demonstrated the linalool capacity against inflammatory processes, oxidative stress, and mitochondrial dysfunction. Linalool modulate the mitochondrial functions through the increase of maximal respiration, spare capacity and phospholipid regulation. These findings suggest that linalool may be a good candidate for further preclinical studies and future translational studies on neurodegenerative diseases.

In our lipidomic approach we detected interesting changes in phospholipids in ischemic stroke, AD and CADASIL in brain samples, CSF and serum that could help to unravel mechanism in these diseases and help to propose different targets based on the evaluation of phospholipid profile.

**Perspectives**

Therapy with natural products has shown great advances for the treatment of several diseases. These natural products can act mostly on a single target or on multiple targets, increasing in this way the benefit that could be produced in patients. The results obtained in this research propose linalool as an effective treatment for AD in a triple transgenic mouse at 21 months and one-month postischemia. It would be interesting to carry out a study to evaluate the preventive potential of this monoterpene in both models. Similarly, in the model of cerebral ischemia, the long-term effect of linalool could be evaluated, since it is known that sequelae of stroke can be observed up to 4 months.
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