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Amerongen, Y.F van; Roy, U.; Spaink, H.P.; Groot, H.J.M. de; Huster, D.; Schiller, J.; Alia, A.

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Zebrafish Brain Lipid Characterization and Quantification by ^{1}H Nuclear Magnetic Resonance Spectroscopy and MALDI-TOF Mass Spectrometry

Yvonne F. van Amerongen,[1] Upasana Roy,[1] Herman P. Spaink,[2] Huub J.M. de Groot,[1] Daniel Huster,[3] Jürgen Schiller,[3] and A. Alia[1,3,4]

Abstract

Lipids play an important role in many neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and Huntington's disease. Zebrafish models for these diseases have been recently developed. The detailed brain lipid composition of the adult zebrafish is not known, and therefore, the representativeness of these models cannot be properly evaluated. In this study, we characterized the total lipid composition of healthy adult zebrafish using ^{1}H nuclear magnetic resonance spectroscopy. A close resemblance of the zebrafish brain composition is shown in comparison to the human brain. Moreover, several lipids involved in the pathogenesis of neurodegenerative diseases (i.e., cholesterol, phosphatidylcholine, docosahexaenoic acid, and further, polyunsaturated fatty acids) are detected and quantified. These lipids might represent useful biomarkers in future research toward human therapies. Matrix-assisted laser desorption–ionization time-of-flight mass spectrometry coupled with high-performance thin-layer chromatography was used for further characterization of zebrafish brain lipids. Our results show that the lipid composition of the zebrafish brain is rather similar to the human brain and thus confirms that zebrafish represents a good model for studying various brain diseases.

Introduction

In recent years, transgenic zebrafish models have been developed for several neurodegenerative diseases, such as Huntington's disease (HD),[1] Parkinson's disease (PD),[2–4] and Alzheimer's disease (AD).[5,6] The availability of several mutants for these diseases results from the fact that zebrafish have a similar brain organization to that of human brains.[5] Brain regions that are affected in neurodegenerative diseases in humans show similar cell type content in zebrafish brains.[7] Moreover, the presence of a dopaminergic system, glia and astrocytes, and the existence of ortholog genes for PD, AD, and HD increase the usefulness of zebrafish as a model for these diseases.[7] In general, the advantages of using the zebrafish model system for research are the relatively low cost of housing and maintenance, the fast reproduction, the transparency and size of the larvae, and the ability to house the larvae in a 96-well plate, which open up the unique opportunity to perform affordable *in vivo* high-throughput drug screening.[1,7]

Polar metabolites in the zebrafish brain have recently been characterized and show high similarities with the composition of the human brain.[8] However, brain lipids of the zebrafish have not yet been investigated at all. The importance of these lipids is evident, since they are involved in multiple underlying mechanisms in the pathogenesis of neurodegenerative diseases, which were comprehensively reviewed in recent literature. For example, in AD, an altered membrane lipid metabolism has been observed, which was linked to the oxidative stress of the plaque forming amyloid β-peptide that raises ceramide and cholesterol levels.[9,10] In addition, membrane damage occurs due to fragmentation and lipid peroxidation of membrane phospholipids (PLs). As a consequence, fragmented polyunsaturated fatty acyl residues of the most abundant of these lipids, phosphatidylcholine (PC), can activate the platelet activating factor receptor and cause inflammation.[11,12] Several lipid molecules associated with lipid peroxidation have also been found to be raised in patients with PD.[13] An important factor in the pathogenesis of PD is oxidative stress caused by free radicals or more generally

[1]Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands.
[2]Institute of Biology Leiden, Leiden University, Leiden, The Netherlands.
[3]Faculty of Medicine, Institute of Medical Physics and Biophysics, University of Leipzig, Leipzig, Germany.
[4]Paul Flechsig Institute for Brain Research, University of Leipzig, Leipzig, Germany.

reactive oxygen species.[14] Moreover, raised levels of polyunsaturated fatty acids (PUFA) in the brain, such as docosahexaenoic acid (DHA), were recently found to stimulate the oligomerization of α-synuclein, the protein that forms aggregates in the nerve cells of PD.[14] Finally, several mutations in lipid regulating proteins could be a cause of susceptibility for developing PD.[15] In addition, in brains of patients with HD, the cholesterol biosynthetic pathway has been impaired in the brain due to decreased translocation of binding proteins to the nucleus.[16]

Thus, brain lipids play a vital role in the pathology and progression of neurodegenerative diseases. These lipids, such as cholesterol, PC, DHA, and other PUFAs, have been recognized as potential biomarkers for brain disease and/or targets for future therapy.[10,16–18] Therefore, it is very important to know the precise lipid composition of the zebrafish brain and whether the lipid composition is similar to the human brain. The representativeness of the above-mentioned models can then be evaluated with regard to future research toward human applications. ^{1}H nuclear magnetic resonance (NMR) represents a useful method to characterize lipid compositions of tissues. It has been applied successfully to the characterization[19,20] and quantification[21] of fish oil contents, particularly, the fatty acyl composition. In addition, polar metabolite characterization in zebrafish brain has been previously performed using 1D and 2D ^{1}H NMR.[8]

In the present study, we characterize zebrafish brain lipids using 1D and 2D ^{1}H NMR spectroscopy and matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF-MS) coupled with high-performance thin-layer chromatography (HPTLC). Although an in-depth characterization could not be achieved due to the high complexity, our results show that the lipid composition of the zebrafish brain is very similar to the human brain.[22] In addition, potential lipid biomarkers of various neurodegenerative diseases, that is, DHA, PC, cholesterol, and PUFAs such as linoleic acid (LA) and α-linolenic acid (ALA), were detected and quantified. Human brain levels of cholesterol, DHA, and PC were compared to the zebrafish brain.

Materials and Methods

Animals

Adult wild-type zebrafish (*Danio rerio*) were maintained according to established rearing procedures, in recirculating aquarium systems.[23] The water temperature was maintained at 28°C with a flow rate of 150 L/min, with 12-h day/12-h night light cycles. The fish were fed twice a day with commercial flake food according to Westerfield.[23] All fish were handled according to the Institutional Animal Care and Use Committee guidelines.

Tissue preparation and extraction

Zebrafish were euthanized, their brains were carefully removed from the skull, and immediately frozen in liquid nitrogen. For the extraction of brain lipids from zebrafish, a modified procedure of Suhartono *et al.* was used.[24] The brains from 10 zebrafish (~70 mg fresh weight) were crushed in 1 mL of methanol:water (1:1, v/v) mixture. Subsequently, 1 mL of chloroform was added. The mixture was then sonicated for 15 min and centrifuged at 5000 rpm at 4°C. After centrifugation, the two layers (lower chloroform layer and upper methanol:water layer) were carefully separated and each was dried individually under nitrogen gas flow at 4°C. The dried chloroform layer containing the lipids was dissolved in 1 mL deuterated chloroform and subsequently filtered using a Millipore filter (Millex-HV 0.45-μm Filter Unit). Tetramethylsilane (TMS, 0.03%) was used as a reference.

NMR spectroscopy

The NMR spectra were recorded at room temperature with a Bruker 600 MHz DMX NMR spectrometer using a 5-mm inverse triple high-resolution probe with an actively shielded gradient coil. The ^{1}H NMR spectra were accumulated with 65,000 data points, a 2-s relaxation delay, and a sweep width of 12.4 kHz; 128 scans were required to obtain a satisfactory signal-to-noise ratio. The free induction decays were weighted with an exponential function (0.09 Hz) before Fourier transformation. Two-dimensional homonuclear ^{1}H-^{1}H experiments were performed using the chemical shift correlated spectroscopic (COSY) sequence. The chemical shifts are relative to TMS.

Data analysis

NMR data analysis was performed using MestReNova software version 6.0.3-5604 (Mestrelab Research S.L., Santiago de Compostela, Spain). The concentrations of the various lipids in the brain extract were determined by comparing the integral peak intensity of the compounds of interest with that of the TMS peak, after correcting for the number of contributing protons and for tissue weight. Quantification of cholesterol and PC was made through the characteristic NMR signal as follows: for cholesterols (i.e., free cholesterol and cholesterol esters) at 0.7 ppm (C_{18}-CH_3); for PC at 3.3 ppm ($-N^+-(CH_3)_3$). Deconvolution was performed by applying a fit region to the 0.85–0.95 ppm range of the spectrum and fitting the peaks one by one to be able to calculate the areas of the individual resonances.

High-performance thin-layer chromatography

The organic brain extract was subjected to HPTLC before MALDI-TOF-MS as described previously.[25] Samples were applied on to HPTLC silica gel 60 plates (10×10 cm^2 with aluminum backs; Merck, Darmstadt, Germany) and developed in vertical TLC chambers using $CHCl_3$–ethanol–water–triethylamine (35/35/7/35, v/v/v/v) as the mobile phase for the separation of PLs. Lipids were visualized by spraying with a solution of primuline.[26] Upon excitation by ultraviolet light (366 nm), individual lipids become detectable as colored spots. These spots were assessed by using a digital image system in combination with the program Argus × 1 delivered by BioStep (Jahnsdorf, Germany).

Coupling of HPTLC with MALDI-TOF-MS

The TLC plates for MS analysis were cut into smaller pieces containing all relevant lipid spots. These pieces were mounted to a prototype MALDI-TOF-MS adapter target[27] with double-sided, conductive adhesive tape (Leit Tabs; Plano GmbH, Wetzlar, Germany). All MALDI spectra of TLC-separated lipids shown in this article were directly obtained from the HPTLC plates, and this approach was described in more detail

earlier.[27] The matrix addition [a 100-mg/mL solution of 2,5-dihydroxybenzoic acid (DHB) in acetonitrile–water (1/1, v/v)] was performed manually on circled spots monitored by previous primuline staining. Selected negative ion mass spectra were recorded in the presence of 9-aminoacridine as recently described.[28]

MALDI-TOF mass spectrometry

All MALDI-TOF mass spectra were acquired using an Autoflex I mass spectrometer (Bruker Daltonics, Billerica, MA) with ion reflector, as described earlier.[25] The system utilizes a pulsed 50 Hz nitrogen laser emitting at 337 nm. The extraction voltage was 20 kV, and gated matrix suppression was applied to prevent the saturation of the detector by matrix ions. All spectra were acquired in the reflector mode using delayed extraction. Spectral mass resolutions and signal-to-noise ratios were determined by the instrument software Flex Analysis 2.4 (Bruker Daltonics). The mass spectrometer was calibrated using the molecular ions of a lipid mixture desorbed from a standard DHB preparation applied next to the spots of interest. Selected measurements were also performed with DHB saturated with CsCl. This minimizes the interferences between different adducts (H^+ and Na^+) and differences in the fatty acyl compositions of the lipids. This procedure is essentially described in Schiller *et al.*[29] In selected cases, postsource decay (PSD) spectra were also recorded to confirm either the headgroup of the corresponding lipid (positive ion mode) or the fatty acyl compositions (negative ion mode).[30]

Result and Discussion

Brain lipids are involved in the pathogenesis of neurodegenerative diseases.[9–16] To characterize the zebrafish brain lipids and assess its similarity to the human brain, we analyzed the total lipid fraction of normal adult zebrafish brain using ^{1}H NMR and MALDI-TOF-MS. Figure 1 shows the representative ^{1}H NMR spectrum of the total lipid extracts prepared from normal zebrafish brain. The main lipid signals

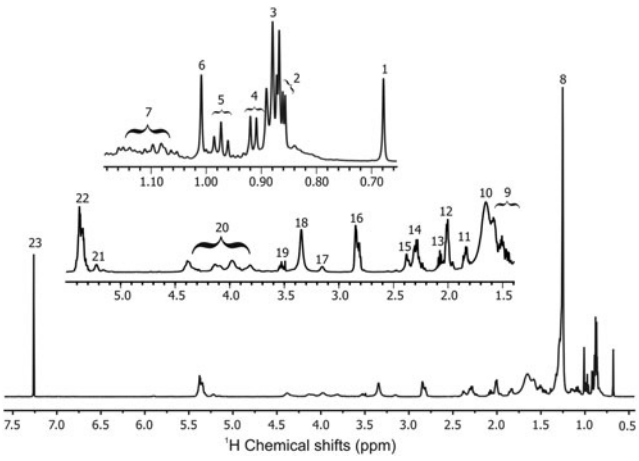

FIG. 1. The ^{1}H nuclear magnetic resonance (NMR) spectrum of the total lipid extract from normal adult zebrafish brain. The ^{1}H chemical shifts were determined relative to tetramethylsilane (at 0.00 ppm) as reference. The assignments of the peak numbers are shown in Table 1.

detected in zebrafish brains are from cholesterol (Chol), the glycerol, and the headgroup of PLs and their fatty acyl residues. The complete assignment of the resonances detectable in the spectrum in Figure 1 is shown in Table 1. The assignment of the zebrafish brain lipid resonances was aided by comparing our spectrum with previous ^{1}H NMR studies using isolated, relevant compounds[31] or with fish (oil),[19,20] which is a rich source of (highly unsaturated) lipids. A comparison of the lipid composition of the zebrafish brain with that of human brain is also shown in Table 1. The overall peak patterns of the lipids in the zebrafish and human brain appear very similar. However, some of the peaks, for example, at chemical shifts 0.880, 0.973, 1.653, 2.075, and 2.370 ppm in the zebrafish brain, were not observed in a previous study of the human brain.[22] Figure 2 shows a 2D homonuclear (^{1}H-^{1}H) dipolar correlation NMR spectrum of the total lipid extract of the normal zebrafish brain. On the basis of the cross peaks, a clear assignment of several brain lipids was obtained. The signals at 0.859 and 0.870 ppm were assigned to the terminal CH_3 groups of cholesterol and at 0.915 ppm to the proton at the C_{21} of cholesterol. The 2D NMR spectrum also confirms the presence of resonances that were not seen in the human brain (Fig. 2; Table 1). An explanation for this difference may be attributed to the prevalence of unsaturated fatty acyl residues in the zebrafish brain as compared to the human brain. According to Tocher *et al.*,[32,33] the most prevalent PUFAs in freshwater fish, such as the zebrafish, are ω-3 fatty acids such as ALA and ω-6 fatty acids such as LA. Both are taken up from nutritional lipids and are, therefore, named essential fatty acids (EFAs).[34] Freshwater fish can convert these EFAs to form highly unsaturated fatty acids (UFAs), such as the ω-3 fatty acids, eicosapentanoic acid, and DHA.[23] Humans can synthesize these fatty acids only in small amounts,[35] so dietary intake is needed. The signal at 0.880 ppm, not seen in the human brain, was assigned to the terminal CH_3 group of an ω-6 fatty acid, most likely LA. Another peak that was not found in the human brain spectrum is the triplet at 0.973 ppm, which can be assigned to the terminal CH_3 of an ω-3 FA, probably ALA. The clear separation of the ω-3 and ω-6 fatty acid methyl resonances can be explained by the fact that the protons of the terminal CH_3 group of ω-3 fatty acids are close to a double bond. This results in a downfield chemical shift of the signal of the methyl group of non-ω-3 fatty acids that can be used for quantification.[21] The DHA signals are well separated from non-DHA fatty acids. In DHA, C_2 and C_3 methylene ($C_{2,3}$-CH_2) are interposed between the carboxyl group at C_1 and the double bond at C_4. Because of this electronegative environment ($-$I effect), the $C_{2,3}$ methylene groups of DHA result in resonances in an isolated downfield region (between 2.32 and 2.42 ppm), well separated from the signals of similar methylene protons of non-DHA fatty acids (2.28 and 2.37 ppm) (Table 1).[21,36]

Surprisingly, a DHA-specific resonance was not reported in the human brain in a previous NMR study,[22] although DHA is one of the most abundant fatty acids in the human brain.[37] A possible explanation for this discrepancy could be that the brain biopsies that were used to characterize normal human brain lipids were isolated from the tissue surrounding the brain tumor.[22] The investigated tumors mostly reside in the white matter and meninges, while DHA is predominantly found in the gray matter.[38] Therefore, the reason why DHA was not detected in the human brain, but was seen in zebrafish

TABLE 1. ^{1}H NMR CHEMICAL SHIFT ASSIGNMENTS OF LIPID IN DEUTERATED CHLOROFORM ($CDCl_3$) EXTRACTED FROM ZEBRAFISH BRAIN[a] AND FROM NORMAL HUMAN BRAIN[b]

			Zebrafish[a]	*Human*[b]
Peak no.	*Assignments*	*Measured at*	*Chemical shift (ppm) 600 MHz*	*Chemical shift (ppm) 250 MHz*
1	Chol (C_{18})	s	0.67	0.67
2	Chol ($C_{26,27}$)	dd	0.85, 0.87	0.83, 0.85
3	ω-6 FA -CH_3 (terminal)	t	0.88	–
4	Chol (C_{21})	d	0.91	0.89
5	ω-3 FA -CH_3 (terminal)	t	0.97	–
6	Chol (C_{19})	s	1.00	0.99
7	Chol	m	1.11	1.10
8	Chol + FA (CH_2)	s	1.25	1.27
9	Chol + FA (CH_2)	m	1.52	1.50
10	FA (CH_2)	bs	1.65	–
11	Chol + FA (CH_2)	d	1.83	1.80
12	FA (CH_2)–Chol	m	2.00	2.00
13	FA (CH_2)	quin	2.07	–
14	FA (CH_2)–Chol	m	2.28	2.27
15	FA (CH_2)–DHA	m	2.37	–
16	FA (CH_2)–PUFA	dd	2.82	2.80
17	PLs	bs	3.15	3.20
18	PC	s	3.34	3.30
19	Chol (C_3)	m	3.52	3.53
U	Unassigned	bs	–	3.63
20	Phosphotidylglycerol	bm	4.08	4.18
21	Sphingolipids Dolichols Plasmalogens	m	5.22	5.20
22	FA (-CH=CH-) and Chol	m	5.36	5.35
23	$CDCl_3$	s	7.26	7.25

[a]This study.
[b]Adapted from Tugnoli *et al.*[22]
s, singlet; d, doublet; t, triplet; m, multiplet; quin, quintet; dd, double doublet; bs, broad singlet; bm, broad multiplet; Chol, cholesterol; DHA, docosahexaenoic acid; FA, fatty acids; PC, phosphatidylcholine, PLs, phospholipids; PUFA, polyunsaturated fatty acid; NMR, nuclear magnetic resonance.

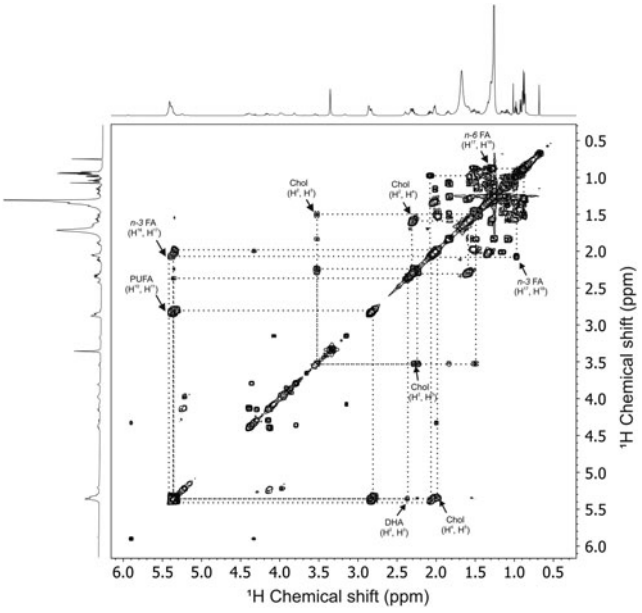

FIG. 2. High-resolution 2D ^{1}H-^{1}H homonuclear correlation spectrum of the total lipid extract from normal adult zebrafish brain.

brain, could be caused by sampling differences rather than its actual absence.

For both the human and zebrafish brain, FA signals with some overlaps with cholesterol signals are observed at 1.25/1.27 and 5.36/5.35 ppm, indicating that further unsaturated fatty acids are detectable in the human tissue.[22] One of the candidates could be oleic acid, the main constituent of myelin, which in turn is the main constituent of the white matter.[39] The human brain signal at 3.63 ppm remained unassigned by Tugnoli *et al.*[22] Whereas this signal does not show up in our spectra, it was seen in an older zebrafish brain lipid sample (data not shown). Two-dimensional COSY NMR of this old lipid fraction from zebrafish brain revealed similarities to cholesterol and FAs (data not shown), indicating a possible breakdown (oxidation) product of these compounds. The remaining two signals that are only seen in zebrafish brain, at chemical shifts 1.653 and 2.075 ppm, were assigned to parts of ω-3 fatty acids (Table 1).

Since PLs are major constituents of the brain,[40] MALDI-TOF-MS coupled with HPTLC was used for further characterization of the zebrafish brain PLs. Figure 3A shows the thin-layer chromatograms of a reference mixture of different, known PLs (Fig. 3-a) and the zebrafish lipid brain extract (Fig. 3-b). A good separation quality of the individual PLs is evident and PC was found to be the most prominent PL in

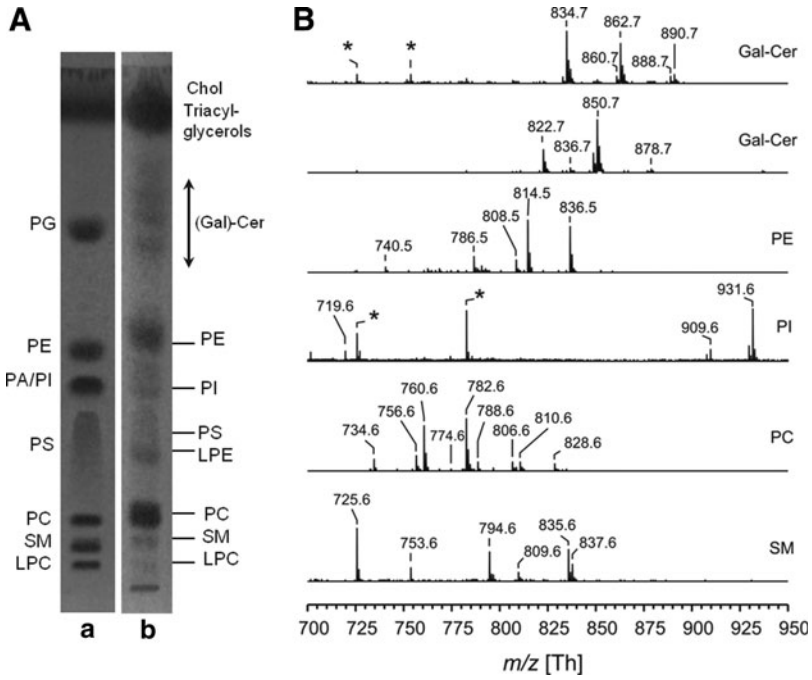

FIG. 3. **(A)** Image of a high-performance thin-layer chromatography (HPTLC) plate of a reference PL mixture (1.69 mg each) **(a)** and the zebrafish lipid brain extract **(b)** subsequent to primuline staining. R_f values of cerebrosides are very similar to the R_f value of the PG as described previously.[40] **(B)** Positive ion matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectra of the individual PL fractions of the zebrafish brain extract recorded directly after HPTLC separation. Even rather pale spots (e.g., the PI spot in **A**) gave high-quality MALDI mass spectra. All peaks are marked according to their *m/z* ratio. For detailed peak assignments, refer to Table 2. SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; (Gal)-Cer, (Galacto)-cerebrosides; PG, phosphatidylglycerol; PL, phospholipid. Unknown impurities in selected lipid fractions are marked by *asterisks* (*). The assignment of the glycolipids was made with reference to a previous article.[40]

the zebrafish brain. Spots corresponding to (galactosyl)-cerebrosides and cholesterol are also clearly detectable and assigned in Figure 3A. Please note that different mass spectra can be obtained in dependence on the position where the laser beam hits the spot on the TLC plate. This is explained by the inhomogeneous distribution of the lipids in dependence on their fatty acyl composition. This effect is more pronounced the higher the R_f value and is thus exemplarily shown for the (galactosyl)-cerebroside fraction.[27] Moderate differences in the migration properties (R_f values) of lipids in the zebrafish brain extract in comparison to the lipid standard are obvious, but these are caused by differences in the fatty acyl compositions of the individual lipids: it is well known that the distribution of the lipids within a given spot is not homogeneous, but depends on the fatty acyl composition and particularly the chain length.[26] These differences can be easily resolved by subsequent mass spectrometric analysis. The positive ion MALDI-TOF mass spectra of some selected brain lipid fractions (obtained directly from the developed TLC plate) are shown in Figure 3B and the assignments of all detected major peaks are provided in Table 2. Both the H^+ and Na^+ adducts were clearly observed in the spectrum of individual PLs, whereby the protons are stemming from the carboxylic group of the used matrix, while sodium ions are omnipresent in all biological samples. Furthermore, methodological details are available in Fuchs.[41] There are interferences between the individual adducts and differences in the fatty acyl compositions: for instance, a PC peak at *m/z* 782.6 might be caused either by the Na^+ adduct of PC 16:0/18:1 or the H^+ adduct of PC 16:0/20:4. Therefore, all ambiguous peak assignments were confirmed by repeating the measurement with CsCl-saturated matrix since this approach enables unequivocal assignments.[29] The representative spectra of PC and phosphatidylethanolamine (PE) fractions measured with the CsCl-saturated matrix and the corresponding assignments are shown in Supplementary Figure S1 (Supplementary Data are available online at www.liebertpub.com/zeb).

TABLE 2. SURVEY OF THE MOST INTENSE PEAKS DETECTED IN THE POSITIVE ION MALDI-TOF MASS SPECTRA OF THE ZEBRAFISH BRAIN LIPID EXTRACT AND THE CORRESPONDING ASSIGNMENTS[a,b]

Peak position (m/z)	*Assignment of molecular mass*
502.3	LPE 18:1 ($+Na^+$)
524.3	LPE 18:1 ($-H^+ +2Na^+$)
551.0	Oligomerization product of DHB matrix
725.5	SM 16:0 ($+Na^+$)
734.6	PC 16:0/16:0 ($+H^+$)
740.5	PE 16:0/18:1 ($+Na^+$)
753.5	SM 18:0 ($+Na^+$)
756.6	PC 16:0/16:0 ($+Na^+$)
760.6	PC 16:0/18:1 ($+H^+$)
782.6	PC 16:0/18:1 ($+Na^+$)
786.5	PE 16:0/22:6 ($+Na^+$)
788.6	PC 18:0/18:1 ($+H^+$)
806.6	PC 16:0/22:6 ($+H^+$)
808.5	PE 16:0/22:6 ($-H^+ +2Na^+$)
810.6	PC 18:0/18:1 ($+Na^+$)
814.5	PE 18:0/22:6 ($+H^+$)
822.7	Gal-Cer 24:1/18:1 ($+Na^+$)
828.6	PC 16:0/22:6 ($+Na^+$)
835.6	SM 24:1 ($+Na^+$)
836.5	PE 18:0/22:6 ($-H^+ +2Na^+$)
850.7	Hydroxylated Gal-Cer 24:1/18:1 ($+Na^+$)
909.6	PI 18:0/20:4 ($+H^+$)
931.6	PI 18:0/20:4 ($+Na^+$)

Spectra were recorded subsequent to separation by HPTLC.

[a]All PL besides SM and PC contain functional groups showing exchange with the solvents and/or ions of the matrix solution leading to complex peak patterns.

[b]Most of the lipid assignments are based on Fuchs *et al.*[40]

Gal-Cer, Galacto-cerebrosides; LPE, lysophosphatidylethanolamine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; HPTLC, high-performance thin-layer chromatography; MALDI-TOF, matrix-assisted laser desorption–ionization time-of-flight.

Table 3. Concentration and Amount of Possible Biomarker Lipids/Unit Wet Weight in Zebrafish Brain

Lipids	*Concentration (μmol/g)*	*Amount (mg/g)*
Cholesterol	22.35 ± 1.64	8.64 ± 0.63
PC	20.37 ± 1.62	15.49 ± 0.51
Fatty acyl composition of PC		
DHA	23.42 ± 1.48	7.69 ± 0.17
PUFA ω-3 LA	20.14 ± 1.32	5.64 ± 0.37
PUFA ω-3 ALA	28.80 ± 0.57	8.01 ± 0.16

ALA, α-linolenic acid; LA, linoleic acid.

Docosahexaenoyl residues are clearly detectable in the PC fraction at *m/z* 806.6 and 828.6 (PC 16:0/22:6) as well as in the PE fraction at *m/z* 814.5 and 836.5 (PE 18:0/22:6) (Fig. 3B). Docosapentaenoyl residues (22:5) are also clearly visible in the PC fraction at *m/z* 808.6 and 830.6 (PC 16:0/22:5), while 22:5 is not detectable in the PE fraction. Information about the fatty acyl residues within the selected PE and PI fractions has been additionally confirmed by negative ion MALDI-TOF mass spectra in conjunction with PSD capability of MALDI-MS (Supplementary Fig. S2). The identification of these fatty acyl residues by MS confirms the results obtained by ^{1}H NMR. A more detailed discussion of all other detectable lipid species was beyond the scope of this article. However, further methodological details are available in Fuchs.[41]

Our results show that lipids that have been proposed in previous studies to be potential biomarkers for neurodegenerative diseases can be detected in zebrafish brain.[10,16–18] These species include (i) cholesterol, which increases in AD and HD; (ii) PC, which decreases in AD; and (iii) DHA/PUFAs, which are raised in PD and suggested to promote the formation of highly soluble oligomers with α-synuclein that precede the insoluble α-synuclein aggregates associated with neurodegeneration. In the ^{1}H NMR spectrum, almost none of the signals of these lipids overlapped with other lipid peaks, except for the peaks in the 0.85–0.95 ppm range. In this study, the signal for the terminal CH_3 of ω-3 overlaps with the cholesterol resonance, which makes quantification more difficult. However, by performing line fitting analysis or deconvolution, peaks could be separated and integrated. The concentrations and amounts of the above-mentioned potential lipid biomarkers were quantified in normal adult zebrafish brain and are shown in Table 3. Table 4 shows the reported concentrations and the amounts of cholesterol, DHA, and PC in human brain. Cholesterol concentration in the adult human brain is reported to be 23 mg/g.[42] However, Igarashi *et al.* found cholesterol amounts of 45 ± 13.9 μmol/g (equal to about 17.4 mg/g) in human brain.[43] The reported amount of DHA in the adult human brain is about 5 g,[44] which results in 3.7 mg/g, when the average adult human brain weight of 1350 g is considered. Igarashi *et al.*[43] reported a DHA concentration in the human brain cortex of 10.23 ± 1.8 μmol/g. The amount of PC in human brain white matter is ~9.37 mg/g and in gray matter ~8.01 mg/g.[45] The concentrations of LA and ALA in the human brain have not been reported so far.

Table 4. Concentration and Amount of Cholesterol, DHA, and PC/Unit Wet Weight of Human Brain

Lipids	*Concentration (μmol/g)*	*Amount (mg/g)*
Cholesterol	45 ± 13.9[a]	± 23.00[b]
PC	–	± 9.37 (WM)[c] ± 8.01 (GM)[c]
DHA	10.23 ± 1.85[a]	± 3.70[d]

[a]Igarashi *et al.*[43].
[b]Dobbing and Sands[42].
[c]Soderberg *et al.*[45]
[d]Martinez.[44]
GM, gray matter; WM, white matter; –, not detectable.

Upon comparing Tables 3 and 4, it is clear that the relative cholesterol concentration is about two times higher in the human brain, while the relative DHA and PC levels are approximately two times lower when compared to the zebrafish brain. Although quantitative differences in lipids between humans and zebrafish brains are seen, all types of lipids, which could be important for future human therapies (such as glycolipids), are also present in the zebrafish brain. When differences in lipid concentrations and amounts are taken into account properly, the zebrafish brain may serve as a valid model for several lipid-related brain diseases, including neurodegenerative diseases.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
A. Alia, PhD
Leiden Institute of Chemistry
Leiden University
Leiden 2300 RA
The Netherlands

E-mail: a.alia@chem.leidenuniv.nl
alia.aliamatysik@medizin.uni-leipzig.de