

Analysis of oxysterols and cholesterol in prefrontal cortex of suicides



Erika Freemantle^{1,2}, Gary Gang Chen², Cristiana Cruceanu^{1,2}, Naguib Mechawar²
and Gustavo Turecki²

¹ Department of Human Genetics, McGill University, Montreal, QC, Canada

² McGill Group for Suicide Studies, Department of Psychiatry, McGill University, Montreal, QC, Canada

Abstract

Brain oxysterol levels, which are enzymatic oxidation products of cholesterol (Chl), have been proposed to reflect the dynamic process of physiological synapse maintenance and repair of nerve terminals within the central nervous system (CNS), due to the turnover of membrane Chl. Modifications of oxysterols have important implications in neurological conditions, especially in neurodegenerative and psychiatric disorders in which alterations of synaptic plasticity or cell signalling are implicated, such as depression. Oxysterols can diffuse across the blood–brain barrier and have been hypothesized to provide a mechanism by which the brain can eliminate excess Chl to maintain a steady state. Relations of 24-hydroxycholesterol (24OH) and 27-hydroxycholesterol (27OH) specifically may provide a depiction of CNS Chl homeostasis. Thus, the objective of this study was to integrate oxysterol measures and gene expression measures in an effort to identify how they may relate to depression and suicide. Using post-mortem human prefrontal cortex tissue, quantification of metabolites by GC–MS and gene expression by qRT-PCR were performed with the aim to provide a characterization of enzymatic oxidative Chl homeostasis. Results show a significant increase in 24OH, which suggests a higher turnover of Chl to 24OH in the prefrontal cortex of suicide cases. An increase in 24OH may, in combination with liver-X receptor activation, explain the observed reduction of low central and peripheral Chl in suicide and would have implications for synapse maintenance and loss in the neuropathology of depression and suicide.

Received 31 August 2012; Reviewed 11 October 2012; Revised 30 October 2012; Accepted 1 December 2012;

First published online 1 February 2013

Key words: Brain lipids, cholesterol, neurodegeneration, oxysterols, suicide.

Introduction

While alterations in lipid and cholesterol (Chl) metabolism have often been reported in major depression and suicidal behaviour (Mossner et al., 2007), it is unclear how these findings relate to the central nervous system (CNS), which is highly enriched in Chl, and whether this may have functional or structural contributions to underlying neuropathology. The association between low levels of peripheral Chl and suicidal and violent behaviour has been shown in a number of studies (Golomb et al., 2000; Coryell and Schlessler, 2007; Atmaca et al., 2008; Boscarino et al., 2009; De Berardis et al., 2009), but in the CNS, limited data exist. We have found a significant decrease in total Chl levels in violent compared to non-violent suicides in the frontal cortex (Lalovic et al., 2007). Another group also reported a significant decrease in Chl in the visual association cortex in subjects with major depressive disorder (Beasley et al., 2005). These studies

complement the association of low Chl levels in psychiatry and suggest a basis for the potential role of Chl within the CNS as a factor in the underlying neuropathology.

Neuronal Chl levels are hypothesized to be shuttled from astrocytes for use in membrane repair and synapse remodelling. This recycling is proposed to be achieved primarily by two mechanisms of (1) lipoprotein mediated transport and (2) enzymatic conversion to oxysterols (reviewed in Pfrieger and Ungerer, 2011). The side chain hydroxylation of sterols renders the molecule more polar (Fig. 1) and thus oxysterols can cross the blood–brain barrier whereas Chl cannot to an appreciable extent (Bjorkhem, 2006).

Oxysterols are implicated in lipid metabolism, myelination and neurodegeneration potentially due to their role as liver-X receptor (LXR) agonists (Wang et al., 2002; Makoukji et al., 2011); the most potent oxysterols are 22-hydroxycholesterol, 24S-hydroxycholesterol (24OH), 24S,25-epoxycholesterol and 27-hydroxycholesterol (27OH; Janowski et al., 1999). Chl is converted to oxysterols via the side-chain hydroxylation and the most abundant Chl derived oxysterol in the CNS is 24OH, mediated by 24-hydroxylase encoded by the gene *CYP46A1*. This gene

Address for correspondence: Dr G. Turecki, McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, 6875 LaSalle Blvd, Montreal, QC H4H 1R3, Canada.
Tel.: (514) 761 6131 Fax: (514) 762 3023
Email: Gustavo.Turecki@McGill.ca

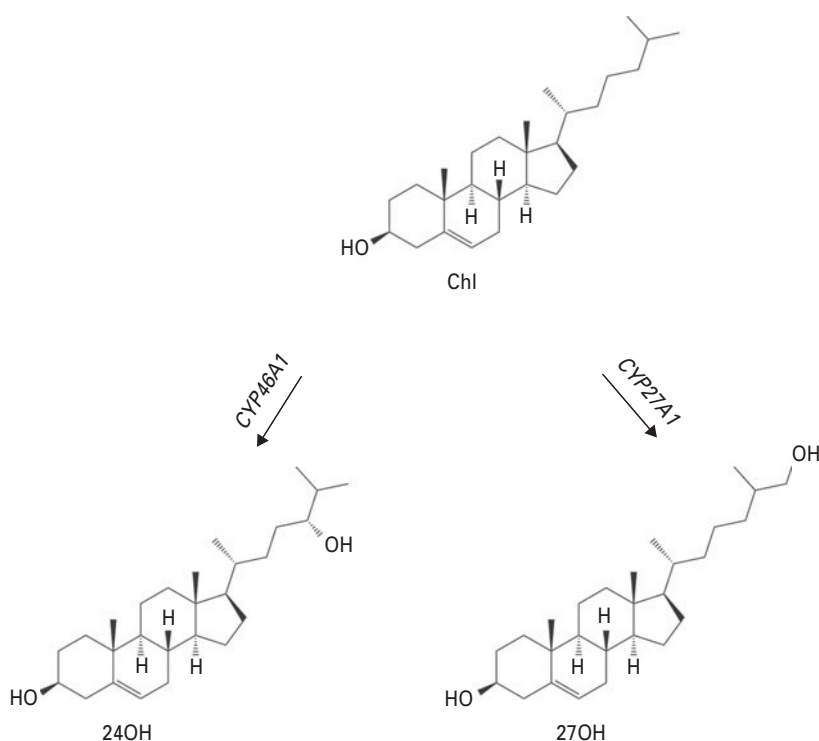


Fig. 1. Side chain enzyme mediated hydroxylation of cholesterol (Chl) to central nervous system oxysterols. Chl is converted to 24-hydroxycholesterol (24OH) and 27-hydroxycholesterol (27OH) by *CYP46A1* and *CYP27A1* respectively.

is highly expressed in neurons and may be expressed in astrocytes and microglia in situations of neurotrauma and neurodegeneration (Brown et al., 2004), likely in order to facilitate disassembled membrane Chl elimination (Cartagena et al., 2008, 2010). 27-hydroxylase is encoded by *CYP27A1* which is largely thought to be expressed by astrocytes, oligodendrocytes and glia, with limited neuronal expression (Brown et al., 2004; Gilardi et al., 2009). 27OH is a major intermediary in the delivery of sterols to the liver, serves as a substrate in the production of bile acids and is much less abundant in the CNS than 24OH. Genetic mutations in *CYP27A1* result in the disorder cerebrotendinous xanthomatosis, a rare autosomal recessive lipid storage disorder characterized by an accumulation of Chl in tendons and the nervous system and is associated with neuronal loss, reactive astrocytosis, neurodegeneration and neurocognitive impairment (Pilo de la Fuente et al., 2008). Thus, while 27OH may be a minor oxysterol in the CNS relative to 24OH, it is likely to have functional importance. *CYP27A1* has also been implicated in amyotrophic lateral sclerosis by a recent genome-wide association study (Diekstra et al., 2012), further implying a role for this oxysterol in neuron survival and neurodegeneration.

24OH is known to be cytotoxic in neuronal cultures by inducing necrosis (Yamanaka et al., 2011). Expression mapping studies have shown that in Alzheimer's disease there was a relative increase in the amount of *CYP46A1* in neurons and neuritic plaques in grey matter and increased levels of *CYP27A1* in white matter (Brown et al.,

2004), suggesting an imbalance of these two oxysterols in neurodegeneration. Efforts to define the relationship between metabolites and gene expression in brain tissue have yielded mixed results (Liao et al., 2011). Thus, the objective of this study was to identify potential alterations in oxysterol conversion and LXR-regulated genes by quantification of oxysterols and expression of oxysterol genes in post-mortem cortical brain tissue of suicides.

Method

Subjects

Brain samples (Brodmann area 47 of the prefrontal cortex) from suicide cases and controls were obtained from the Quebec Suicide Brain Bank (QSBB; Douglas Institute; www.douglas.qc.ca/suicide). Approval for this study was granted by the Douglas Hospital Institutional Review Board in accordance with the 1964 Declaration of Helsinki and written informed consent was obtained from each participating family. Cause of death was ascertained by the Quebec Coroner's Office. All deaths were sudden and without prolonged agonal state and none of the samples analysed in this study had evidence of neuropathological illness. Sociodemographic data as well as clinical information, including psychiatric and medical history, were obtained through psychological autopsies consisting of structured interviews with one or more informants of the deceased, supplemented by a review of coroner's notes and medical records, as described

Table 1. Subject characteristics

	Con (<i>n</i> =8)	S (<i>n</i> =14)	<i>p</i> value
pH	6.5±0.25	6.7±0.23	0.221
Age	37±12	43±12	0.296
PMI	24±6	27±7	0.264
RIN	5.8±0.55	5.8±0.56	0.938
Cause of death			
Car accident	3	–	–
Cardiac related	5	–	–
Hanging	–	12	–
Drowning	–	1	–
Gun shot	–	1	–
Diagnosis of MDD	–	10	–

Con, Controls; S, suicides; PMI, post-mortem interval; RIN, RNA integrity number; MDD, major depressive disorder. Values are mean±s.d.

Con and S do not differ in terms of age, brain tissue pH, PMI or RIN.

in detail elsewhere (Kim et al., 2003; McGirr et al., 2007). This information, including Structured Clinical Interview for DSM-IV Axis I data, was assessed by a clinical panel to obtain best-consensus DSM-IV Axis I diagnoses. Demographic and subject characteristics are listed in Table 1.

Chemicals and materials

All solvents, unless specified otherwise, were obtained from Sigma (USA) and were of analytical grade. 24OH (5-cholestene-3 β ,24S-diol) and 27OH (5-cholestene-3 β ,27-diol) as well as deuterated (d6)-24OH and d6-27OH were purchased from Avanti Polar Lipids (USA). Trimethylsilyl agent *O,N*-bis-(trimethylsilyl) acetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Supelco (Sigma-Aldrich, USA).

Brain tissue processing

Upon arrival at the QSBB, tissues were immediately snap-frozen and stored at –80 °C until further manipulation. This study focused on Brodmann area 47, a region known to be associated with mood disorders and suicidal behaviour and previously implicated in alterations in Chl metabolism in suicides (Lalovic et al., 2007).

Extraction of oxysterols from post-mortem brain

Folch extraction and saponification

Human post-mortem brain tissue from Brodmann area 47 grey matter of ~20–70 mg was transferred to glass vials with 2 ml Folch solvent [chloroform/methanol (MeOH), 2:1, v/v] containing 0.01% butylated hydroxytoluene followed by adding 1000 ng d6-24OH and 100 ng d6-27OH as internal standards (IS). The

homogenization and extraction was carried out at room temperature (RT). The extracts were evaporated under a stream of N₂ then dry residue was dissolved in 1 ml hydrolysis solution containing 10% KOH in MeOH and subjected to an overnight saponification at RT. Following saponification, the hydrolysed solution was extracted with 0.5 ml distilled H₂O and 2 ml diethyl ether. The ether phase from extraction was washed twice with distilled H₂O then dried under a stream of N₂ at RT. The dry residues were dissolved in 1 ml MeOH/H₂O (90:10). After centrifugation at 5000 rpm (2400 g) for 15 min, the liquid phase was transferred into a fresh tube for a further purification by C18 column in order to remove most Chl and other lipids interfering with gas chromatography–mass spectroscopy (GC–MS) analysis of oxysterols.

C18 reverse phase extraction

The Agilent Bound Elute C18 (500 mg, 6 ml) column was equilibrated with MeOH/H₂O (90:10). The liquid phase sample from Folch extraction and saponification was loaded onto the column. The flow-through, containing mainly oxysterols, was passed through a second C18 column to go through a second reverse phase purification process to further remove Chl and other lipid contaminants.

Derivatization

Preparation of oxysterol extracts for GC–MS analysis was performed by initial drying of C18 reverse phase extracted samples. The dry residue was reconstituted with 1 ml MeOH, vortex mixed for 30 s and transferred to a glass conical reaction vial. The samples were then evaporated to dryness and reconstituted with 25 μ l ethyl acetate and vortex mixed. The fractions to be analysed were converted to their trimethyl-silylated derivatives by heating with 100 μ l BSTFA containing 1% TMCS at 70 °C for 45 min. The solvent and reagents were removed under a stream of N₂ at 60 °C to dryness. The residue was dissolved in 200 μ l hexane and the clear hexane phase was transferred into a glass vial, suitable for GC–MS injection.

Gas chromatography–mass spectrometry

Calibration curve preparation

As IS, d6-24OH and d6-27OH were used in all sample analysis and calibration curve preparations. Calibration curves were prepared with a fixed amount of each deuterated IS (d6-24OH and d6-27OH) and increasing amounts of each non-deuterated authentic compound in Folch solvent (chloroform/MeOH, 2:1, v/v) used for brain tissue extraction. Calibration samples were treated and analysed as the experimental samples. Concentrations were calculated on the basis of the slope of the standard curve and on the peak area ratio

(non-deuterated oxysterol:IS) found in the sample. The assay results were linear ($r > 0.99$) in the tested ranges 10–100 ng/mg of wet tissue or solvent for both 24OH and 27OH.

Conditions for gas chromatography–mass spectrometry

The instrument used was an Agilent bench-top Hewlett-Packard 6890/Mass Spectrometer Detector 5973N Chemstation system (Agilent Technologies Inc., USA) equipped with a Hewlett-Packard Automatic Liquid Sampler. The derivatized oxysterols (2 μ l) were injected into a Hewlett-Packard 5890 GC–MS in the splitless injection mode. The injector temperature was held at 280 °C throughout the analysis. Separation of oxysterols was achieved through an HP-5MS capillary column (30 \times 0.25 m, 0.25 μ m phase thickness; J & W Scientific, USA) using helium as carrier gas at a flow rate of 1 ml/min and a column temperature programme (180–310 °C, 20 °C/min) with a final bake out hold of 5 min at 300 °C. The spectrometer was set at 70 eV ion energy, 0.1 mA emission current, 280 °C transfer line temperature and ion source temperature of 225 °C. For electron impact ionization (EI), a mass spectral scan from 50 to 700 m/z was employed for full scan studies. In order to enhance sensitivity for detection under EI mode, selected ion monitoring (SIM) mode was used recording molecular ions at 456 m/z for 27OH and 462 m/z for d6-27OH. In the case of 24OH and d6-24OH, due to the lack of their molecular ions, their most abundant fragment ions at 145 m/z for 24OH and 151 m/z for d6-24OH respectively, were monitored and used for the quantification process. A total of four ions were monitored in SIM EI mode for peak verification and quantification purposes with solvent delay set at 5 min. The retention time for each oxysterol derivative was obtained using the retention times from the calibration curves as reference. Samples were run in triplicate and values derived from calibration curves are expressed as ng analyte/mg wet weight tissue.

Gene expression quantification

RNA was extracted from snap-frozen prefrontal cortical (Brodmann area 47) brain tissue using the Qiagen RNeasy lipid kit including DNase I incubation to remove contaminating genomic sequences. RNA quality was assessed by bioanalyser (Agilent Technologies) and RNA integrity numbers > 5 were deemed satisfactory. Synthesis of complementary DNA was performed with M-MLV reverse transcriptase (Gibco, Canada) with oligo(dT)16 primers (Invitrogen, USA). To quantify expression of RefSeq mRNA, primers of 20 nucleotides in length were designed using Primer3 to produce amplicons of approximately 200 bp for analysis by SYBR Green quantitative real time PCR (qRT-PCR); amplicons of this length have been shown to be ideal for most gene

expression analysis (Fleige and Pfaffl, 2006). Primers (Integrated DNA Technologies, USA) were designed to span an exon junction to obviate genomic contamination. Primer sequences are available upon request. Samples were run in quadruplicate using standard qRT-PCR conditions and Perfecta SYBR Green PCR Master Mix (VWR) on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, USA). Relative expression was calculated using the relative quantification ($\Delta\Delta C_t$) method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous control in the RQ Manager 1.2 software. Expression values are presented as relative quantification values throughout the paper and they represent $2^{-\Delta\Delta C_t}$ metrics in reference to a pooled calibrator sample.

Statistical analysis

Exploratory data analysis method was employed for all data, which reports several measures of skewness and distribution, median, mean and quartiles, making no *a priori* assumptions about data normality. Data normality was then verified using Shapiro–Wilk tests. Outliers were defined as > 2 standard deviations from the group stratified means, detected by boxplots and excluded from analyses. Statistical significance of the analytes measured was compared depending on their distribution. For normally distributed data one-way analysis of variance (ANOVA) was performed. For non-parametric analysis Mann–Whitney *U* tests were performed. For correlational measures, Pearson's test was used for normally distributed data while Spearman's test was used for non-parametric data. For possible confounders such as age, brain pH, post-mortem interval (PMI) and substance abuse, analysis of covariance (ANCOVA) were performed with permutation test corrections based on 1000 permutations. Significance was set at $p \leq 0.05$. Analyses were carried out using SPSS version 15.0 and Prism GraphPad version 5.

Results

Groups did not differ in terms of brain tissue pH, PMI, RNA integrity number or age (Table 1). In suicides, there was a significant increase in 24OH compared to controls with a mean difference of +21 ng/mg tissue (Fig. 2, $p = 0.003$, corrected $p = 0.0017$). While there was no statistically significant difference in the ratio of 24OH or 27OH to Chl in suicides compared to controls, the data suggested an increased conversion of Chl to both 24OH and 27OH oxysterols in suicides compared to controls ($p = 0.075$ and $p = 0.069$ for 24OH and 27OH respectively, Fig. 2). Age did not correlate with any of the measures; however, as alterations in 24OH have been found with ageing, to rule out a potential interaction effect, multivariate models including interaction terms with age were built (corrected model $p = 0.04$, estimate of effect size = 0.302, p -value for group = 0.014, estimate of effect

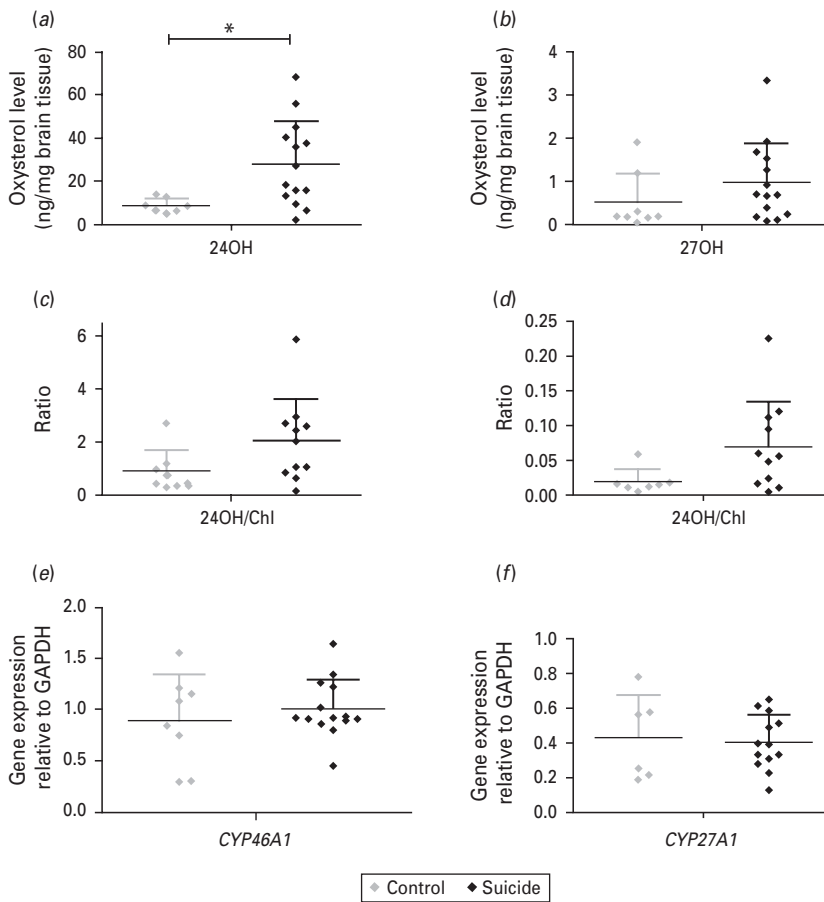


Fig. 2. Levels of oxysterols [24-hydroxycholesterol (24OH) and 27-hydroxycholesterol (27OH); ng/mg brain tissue] and mRNA expression of hydroxylases in post-mortem human brain tissue. Significantly higher levels of 24OH were found in suicides compared to controls (*a*, * $p < 0.05$). No significant differences were found by group for 27OH (*b*) the conversion ratios of oxysterols to cholesterol (Chl; *c* and *d*) or expression of oxysterol conversion enzymes (*e* and *f*). GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

size for group = 0.291, p -value for age = 0.270, estimate of effect size for age = 0.067).

The expression of either *CYP46A1* or *CYP27A1* was not altered (Fig. 2) and while correlations of expression measures and metabolites were in the expected direction, there did not appear to be a discernible relationship between enzyme gene expression and oxysterol levels (Fig. 3).

However there was a trend for a negative relationship between gene expression of oxysterol metabolising enzymes (Fig. 3; $r = -0.430$, $p = 0.066$). To determine if there was a potential difference in expression in relation to brain matter, expression of oxysterol conversion enzymes was assessed separately in grey matter and white matter. *CYP46A1* shows similar expression in both tissue types and *CYP27A1* is more highly expressed in white matter compared to grey matter (Fig. 4). *CYP46A1* expression shows a negative correlation with *CYP27A1* in grey matter and white matter (grey matter Spearman coefficient = -0.430 , $p = 0.066$, white matter Spearman coefficient = -0.800 , $p = 0.0003$; supplementary Fig. S1).

To rule out a potential effect, GAPDH gene expression was evaluated; endogenous GAPDH was not significantly different between groups nor did it impact the results of *CYP* gene expression as assessed by multivariate models including GAPDH expression in either grey matter (mean \pm s.d. 2.50 ± 1.16 and 2.76 ± 1.58 , means test $p = 0.662$; *CYP46A1* corrected model $p = 0.565$, estimate of effect size = 0.054; *CYP27A1* corrected model $p = 0.326$, estimate of effect size = 0.025) or in white matter (mean \pm s.d. 3.73 ± 1.61 and 1.93 ± 0.58 , means test $p = 0.108$; *CYP46A1* corrected model $p = 0.467$, estimate of effect size = 0.033; *CYP27A1* corrected model $p = 0.607$, estimate of effect size = 0.054).

Finally, to investigate whether expression of genes known to be induced by oxysterols were altered in association with increased 24OH, apolipoprotein E (ApoE) and lipoprotein lipase (LPL) expression was assessed in grey and white matter; as such, there was no change in the expression of the LXR-regulated genes ApoE or LPL in either grey or white matter (Fig. 4).

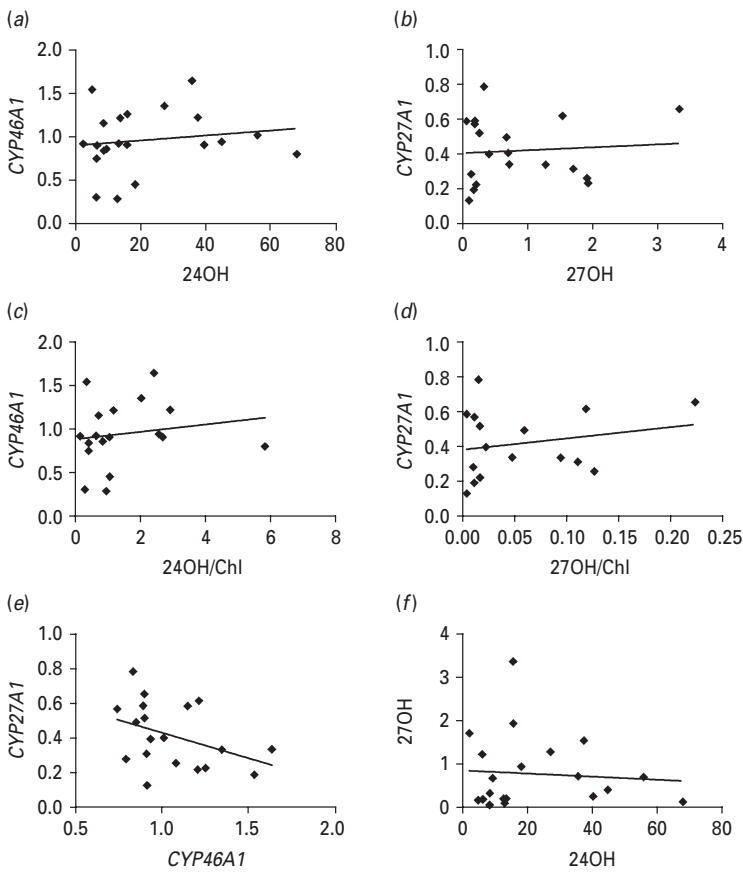


Fig. 3. Assessment of the relationship between 24- and 27-hydroxysterols (24OH and 27OH) gene expression with gene function. There was no discernible relationship between metabolite and expression measures (a–d); however, there is a trend for a negative relationship between gene expression of oxysterol metabolising enzymes (*CYP46A1* and *CYP27A1*; e), while no relationship is observed for oxysterol levels (f). Chl, Cholesterol.

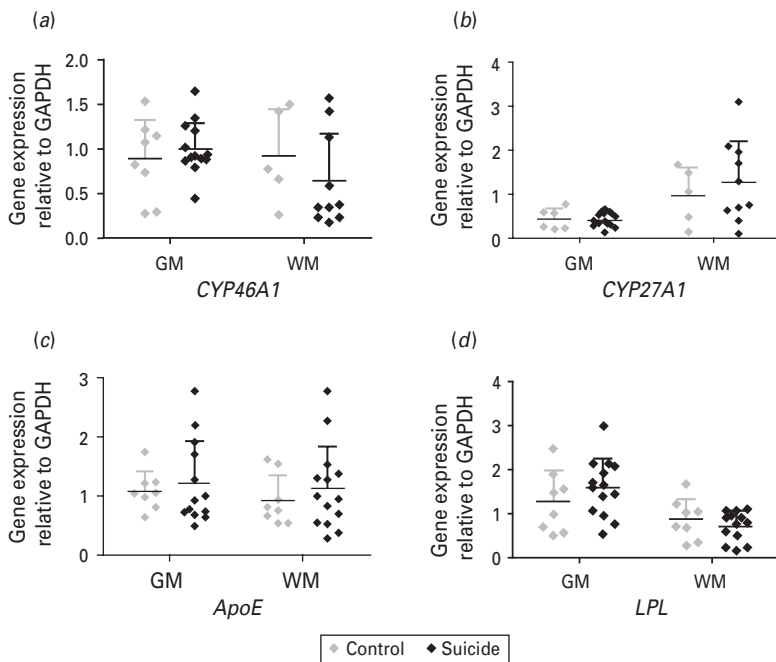


Fig. 4. Expression of hydroxylases and liver-X receptor (LXR) regulated genes in grey (GM) and white matter (WM). No relationship between increased oxysterol levels between suicides compared to controls for genes *CYP46A1* (a) and *CYP27A1* (b) or LXR regulated genes apolipoprotein E (*ApoE*; c) and lipoprotein lipase (*LPL*; d). GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

Discussion

Given the previous associations of lowered Chl levels in psychopathology *vs.* control populations particularly in grey matter (Beasley et al., 2005; Lalovic et al., 2007), this study was initiated to determine if potential increases in the conversion of Chl to the more polar derivatives 24OH and 27OH might play a role in the neuropathology of depression and in suicide. Overall, there was a significant increase in the levels of 24OH, suggesting an increased conversion of Chl to 24OH which is in agreement with the aforementioned results.

It is thought that levels of 24OH reflect release of Chl from membranes in neurodegeneration and neuroplasticity, as there is a significant correlation between 24OH in plasma and neural activity; however, the relationship between plasma levels and levels of oxysterols in brain tissue is unclear (Leoni, 2009; Solomon et al., 2009). The results of this study suggest that increased oxysterol levels in the CNS are also implicated in the neuropathology of depression and suicide.

The literature also suggests that there is an increase of 24OH during ageing (Lutjohann et al., 1996); in this sample cohort, however, there was no association between age and any of the measures. Furthermore, including age as a covariate did not have an effect on the significance of the findings, which may be explained by the fact that the subjects in this study were of comparable age.

As well as metabolite levels, expression levels of *CYP46A1* and *CYP27A1* have been linked to neurodegenerative disorders and thus, an additional objective of this study was to examine potential deregulation in genes encoding the molecules responsible for the enzymatic conversion of Chl by *CYP46A1* and *CYP27A1* to their respective oxysterols, 24OH and 27OH. While no discernible relationship between expression and metabolites was found, a relationship cannot be discounted as the half-lives of the enzymes are not known, and it would potentially be of interest for follow up studies to quantify enzyme levels directly. While the hypothesis of this study was a change in turnover of Chl to oxysterol metabolites specifically in grey matter in suicides, the contribution of different tissue types in expression of oxysterol genes in human brain is largely unknown. As the impact of brain cell type in oxysterol regulation is of essential importance and it has been suggested that there could be an imbalance of expression in different cell types in neuropathology (Brown et al., 2004), gene expression was evaluated in both grey matter and white matter. Interestingly, *CYP46A1* showed comparable levels in both grey and white matter, whereas *CYP27A1* showed increased expression in white matter over grey matter, suggesting an oligodendrocyte enrichment in the prefrontal cortex. Overall, in both grey matter and white matter tissue types, the data suggest that there is a negative relationship between the expression of *CYP46A1* and *CYP27A1*,

potentially indicating a differential regulation of oxysterol conversion enzymes in grey matter and white matter.

This is, to our knowledge, the first investigation of the distinct expression patterns of Chl-related genes in grey and white matter. Given that 24OH and Chl may be related structurally to grey matter and white matter volumes (Solomon et al., 2009), it could be of interest for follow-up studies to measure oxysterols in both tissue types. This analysis is more complex, though, as Chl is highly enriched in white matter and would require specialized extraction techniques for white matter oxysterol analysis by GC-MS.

As oxysterols are LXR agonists and 24OH has been shown to directly bind LXRs (Janowski et al., 1996; Willson et al., 1996) mediating dimerization with co-factors and induction of expression of genes via response elements in promoter sequences related to maintenance of lipid trafficking (Abildayeva et al., 2006), it was of interest to determine whether increased levels of oxysterols conferred an increase in the expression of two LXR-regulated genes, *LPL* and *ApoE*. *LPL* and *ApoE* have been proposed to be involved in synapse remodelling (Blain et al., 2004; Xian et al., 2009; Oh et al., 2010) and their expression may be induced by increased oxysterol levels (Wang et al., 2008; Oh et al., 2010). The expression of these genes was unchanged in relation to oxysterol levels; however, the genes require co-activation by LXRs in concert with other regulatory molecules, such as retinoid X receptors (RXRs) and it is possible that increased 24OH is not alone sufficient to induce expression of lipid genes. In astrocyte cell culture, 24OH is known to increase *ApoE*-dependent Chl efflux (Abildayeva et al., 2006). However, it was recently shown in an animal model study that increasing the expression of 24OH with a concomitant increase in 24OH did not result in differentially expressed LXR-regulated genes (Shafaati et al., 2011). Regardless, it remains a possibility that other genes also known to be under the control of oxysterols/LXR signalling, such as inflammatory mediators, are being induced (Bensinger and Tontonoz, 2008; Bensinger et al., 2008) and further research into this area is warranted given the proposed role of inflammation in depression and suicide (Gardner and Boles, 2011; Torres-Platas et al., 2011).

Finally, the relationship between central and peripheral Chl is difficult to determine; however, given that both low peripheral Chl and low central Chl have been observed in depression and suicidal behaviour, an increase in 24OH could potentially provide the link between central and peripheral levels. 24OH has been shown to cross the blood-brain barrier into peripheral circulation. Thus, by mediating LXR activation peripherally, 24OH in combination with nuclear receptor co-factors such as RXRs could induce the expression of genes in Chl regulation and elimination. This would include transport molecules such as ABCA1 and

lipoprotein mediated reverse Chl transport and also CYPs responsible for the conversion of Chl to bile acids. As such, increased 24OH, in addition to influencing neuroplasticity, could provide a key mechanism by which Chl removal from circulation would result in lowered circulating Chl in suicides.

Conclusion

Oxysterols are thought to be involved in membrane Chl recycling and synapse remodelling. A better understanding of brain oxysterols is of importance in the context of both synapse plasticity and maintenance and synapse loss in neurodegeneration. Overall, the results of this study suggest that there is an increase in turnover of Chl to 24OH in the prefrontal cortex of suicides and this could have implications for synapse maintenance and loss in the neuropathology of depression and suicide.

Acknowledgements

The authors thank Dr O. Mamer, McGill University Health Care Centre for excellent GC-MS technical discussions. The Québec Suicide Brain Bank is supported in part by the Fonds de la Recherche Québec Santé (FRQS) through the Réseau Québécois de Recherche sur le Suicide. This work was supported in part by the Canadian Institute of Health Research (CIHR) MOP 53321 to G. T. E. F. received a scholarship from FRQS. C. C. received a scholarship from CIHR. G. T. is an FRQS chercheur boursier. N. M. is a CIHR New Investigator.

Statement of Interest

None.

Supplementary material

For supplementary material accompanying this paper, visit <http://dx.doi.org/10.1017/S1461145712001587>

References

- Abildayeva K, Jansen PJ, Hirsch-Reinshagen V, Bloks VW, Bakker AH, Ramaekers FC, de Vente J, Groen AK, Wellington CL, Kuipers F, Mulder M (2006) 24(S)-hydroxycholesterol participates in a liver X receptor-controlled pathway in astrocytes that regulates apolipoprotein E-mediated cholesterol efflux. *J Biol Chem* 281:12799–12808.
- Atmaca M, Kuloglu M, Tezcan E, Ustundag B (2008) Serum leptin and cholesterol values in violent and non-violent suicide attempters. *Psychiatry Res* 158:87–91.
- Beasley CL, Honer WG, Bergmann K, Falkai P, Lutjohann D, Bayer TA (2005) Reductions in cholesterol and synaptic markers in association cortex in mood disorders. *Bipolar Disord* 7:449–455.
- Bensinger SJ, Bradley MN, Joseph SB, Zelcer N, Janssen EM, Hausner MA, Shih R, Parks JS, Edwards PA, Jamieson BD, Tontonoz P (2008) LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell* 134:97–111.
- Bensinger SJ, Tontonoz P (2008) Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature* 454:470–477.
- Bjorkhem I (2006) Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. *J Intern Med* 260:493–508.
- Blain JF, Paradis E, Gaudreault SB, Champagne D, Richard D, Poirier J (2004) A role for lipoprotein lipase during synaptic remodeling in the adult mouse brain. *Neurobiol Dis* 15:510–519.
- Boscarino JA, Erlich PM, Hoffman SN (2009) Low serum cholesterol and external-cause mortality: potential implications for research and surveillance. *J Psychiatr Res* 43:848–854.
- Brown III J, Theisler C, Silberman S, Magnuson D, Gottardi-Littell N, Lee JM, Yager D, Crowley J, Sambamurti K, Rahman MM, Reiss AB, Eckman CB, Wolozin B (2004) Differential expression of cholesterol hydroxylases in Alzheimer's disease. *J Biol Chem* 279:34674–34681.
- Cartagena CM, Ahmed F, Burns MP, Pajoohesh-Ganji A, Pak DT, Faden AI, Rebeck GW (2008) Cortical injury increases cholesterol 24S hydroxylase (Cyp46) levels in the rat brain. *J Neurotrauma* 25:1087–1098.
- Cartagena CM, Burns MP, Rebeck GW (2010) 24S-hydroxycholesterol effects on lipid metabolism genes are modeled in traumatic brain injury. *Brain Res* 1319:1–12.
- Coryell W, Schlessler M (2007) Combined biological tests for suicide prediction. *Psychiatry Res* 150:187–191.
- De Berardis D, Conti CM, Serroni N, Moschetta FS, Carano A, Salerno RM, Cavuto M, Farina B, Alessandrini M, Janiri L, Pozzi G, Di Giannantonio M (2009) The role of cholesterol levels in mood disorders and suicide. *J Biol Regul Homeost Agents* 23:133–140.
- Diekstra FP *et al.* (2012) Mapping of gene expression reveals CYP27A1 as a susceptibility gene for sporadic ALS. *PLoS ONE* 7:e35333.
- Fleige S, Pfaffl MW (2006) RNA integrity and the effect on the real-time qRT-PCR performance. *Mol Aspects Med* 27:126–139.
- Gardner A, Boles RG (2011) Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 35:730–743.
- Gilardi F, Viviani B, Galmozzi A, Boraso M, Bartesaghi S, Torri A, Caruso D, Crestani M, Marinovich M, de Fabiani E (2009) Expression of sterol 27-hydroxylase in glial cells and its regulation by liver X receptor signaling. *Neuroscience* 164:530–540.
- Golomb BA, Stattin H, Mednick S (2000) Low cholesterol and violent crime. *J Psychiatr Res* 34:301–309.
- Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, Mangelsdorf DJ (1999) Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. *Proc Natl Acad Sci USA* 96:266–271.
- Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ (1996) An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature* 383:728–731.

- Kim C, Lesage A, Seguin M, Chawky N, Vanier C, Lipp O, Turecki G (2003) Patterns of co-morbidity in male suicide completers. *Psychol Med* 33:1299–1309.
- Lalovic A, Levy E, Luheshi G, Canetti L, Grenier E, Sequeira A, Turecki G (2007) Cholesterol content in brains of suicide completers. *Int J Neuropsychopharmacol* 10:159–166.
- Leoni V (2009) Oxysterols as markers of neurological disease—a review. *Scand J Clin Lab Invest* 69:22–25.
- Liao WL, Heo GY, Dodder NG, Reem RE, Mast N, Huang S, Dipatre PL, Turko IV, Pikuleva IA (2011) Quantification of cholesterol-metabolizing P450s CYP27A1 and CYP46A1 in neural tissues reveals a lack of enzyme-product correlations in human retina but not human brain. *J Proteome Res* 10:241–248.
- Lutjohann D, Breuer O, Ahlberg G, Nennesmo I, Siden A, Diczfalusy U, Bjorkhem I (1996) Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. *Proc Natl Acad Sci USA* 93:9799–9804.
- McGirr A, Paris J, Lesage A, Renaud J, Turecki G (2007) Risk factors for suicide completion in borderline personality disorder: a case-control study of cluster B comorbidity and impulsive aggression. *J Clin Psychiatry* 68:721–729.
- Makoukji J, Shackleford G, Meffre D, Grenier J, Liere P, Lobaccaro JM, Schumacher M, Massaad C (2011) Interplay between LXR and Wnt/beta-catenin signaling in the negative regulation of peripheral myelin genes by oxysterols. *J Neurosci* 31:9620–9629.
- Mossner R, Mikova O, Koutsilieri E, Saoud M, Ehli AC, Muller N, Fallgatter AJ, Riederer P (2007) Consensus paper of the WFSBP task force on biological markers: biological markers in depression. *World J Biol Psychiatry* 8:141–174.
- Oh JY, Nam YJ, Jo A, Cheon HS, Rhee SM, Park JK, Lee JA, Kim HK (2010) Apolipoprotein E mRNA is transported to dendrites and may have a role in synaptic structural plasticity. *J Neurochem* 114:685–696.
- Pfriefer FW, Ungerer N (2011) Cholesterol metabolism in neurons and astrocytes. *Prog Lipid Res* 50:357–371.
- Pilo de la Fuente B, Ruiz I, Lopez de Munain A, Jimenez-Escrig A (2008) Cerebrotendinous xanthomatosis: neuropathological findings. *J Neurol* 255:839–842.
- Shafaati M, Olin M, Bavner A, Pettersson H, Rozell B, Meaney S, Parini P, Bjorkhem I (2011) Enhanced production of 24S-hydroxycholesterol is not sufficient to drive liver X receptor target genes *in vivo*. *J Intern Med* 270:377–387.
- Solomon A, Leoni V, Kivipelto M, Besga A, Oksengard AR, Julin P, Svensson L, Wahlund LO, Andreassen N, Winblad B, Soyninen H, Bjorkhem I (2009) Plasma levels of 24S-hydroxycholesterol reflect brain volumes in patients without objective cognitive impairment but not in those with Alzheimer's disease. *Neurosci Lett* 462:89–93.
- Torres-Platas SG, Hercher C, Davoli MA, Maussion G, Labonte B, Turecki G, Mechawar N (2011) Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. *Neuropsychopharmacology* 36:2650–2658.
- Wang L, Schuster GU, Hultenby K, Zhang Q, Andersson S, Gustafsson JA (2002) Liver X receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. *Proc Natl Acad Sci USA* 99:13878–13883.
- Wang Y, Muneton S, Sjoval J, Jovanovic JN, Griffiths WJ (2008) The effect of 24S-hydroxycholesterol on cholesterol homeostasis in neurons: quantitative changes to the cortical neuron proteome. *J Proteome Res* 7:1606–1614.
- Willson TM, Lehmann JM, Kliewer SA (1996) Discovery of ligands for the nuclear peroxisome proliferator-activated receptors. *Ann N Y Acad Sci* 804:276–283.
- Xian X, Liu T, Yu J, Wang Y, Miao Y, Zhang J, Yu Y, Ross C, Karasinska JM, Hayden MR, Liu G, Chui D (2009) Presynaptic defects underlying impaired learning and memory function in lipoprotein lipase-deficient mice. *J Neurosci* 29:4681–4685.
- Yamanaka K, Saito Y, Yamamori T, Urano Y, Noguchi N (2011) 24(S)-hydroxycholesterol induces neuronal cell death through necroptosis, a form of programmed necrosis. *J Biol Chem* 286:24666–24673.