Neurochemistry International 102 (2017) 33–56

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Global gene expression changes in the prefrontal cortex of rabbits with hypercholesterolemia and/or hypertension

Sau-Yeen Loke [a], Peter Tsun-Hon Wong [b], Wei-Yi Ong [a, c, *]

[a] Department of Anatomy, National University of Singapore, 119260, Singapore
[b] Department of Pharmacology, National University of Singapore, 119260, Singapore
[c] Neurobiology and Ageing Research Program, Life Sciences Institute, National University of Singapore, 119260, Singapore

ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form
14 November 2016
Accepted 23 November 2016
Available online 25 November 2016

Keywords:
Cholesterol
Hypertension
Proteasome
Insulin
Histone
Inflammation

ABSTRACT

Although many studies have identified a link between hypercholesterolemia or hypertension and cognitive deficits, till date, comprehensive gene expression analyses of the brain under these conditions is still lacking. The present study was carried out to elucidate differential gene expression changes in the prefrontal cortex (PFC) of New Zealand white rabbits exposed to hypercholesterolemia and/or hypertension with a view of identifying gene networks at risk. Microarray analyses of the PFC of hypercholesterolemic rabbits showed 850 differentially expressed genes (DEGs) in the cortex of hypercholesterolemic rabbits compared to controls, but only 5 DEGs in hypertensive rabbits compared to controls. Up-regulated genes in the PFC of hypercholesterolemic rabbits included CIDEC, ODF2, RNASEL, FSHR, CES3 and MAB21L3, and down-regulated genes included FAM184B, CUL3, LOC100351029, TMEM109, LOC100357097 and PFDN5. Comparison with our previous study on the middle cerebral artery (MCA) of the same rabbits showed many differentially expressed genes in common between the PFC and MCA, during hypercholesterolemia. Moreover, these genes tended to fall into the same functional networks, as revealed by IPA analyses, with many identical node molecules. These include: proteasome, insulin, Akt, ERK1/2, histone, IL12, interferon alpha and NFκB. Of these, PSMB4, PSMD4, PSMG1 were chosen as representatives of genes related to the proteasome for verification by quantitative RT-PCR. Results indicate significant downregulation of all three proteasome associated genes in the PFC. Immunostaining showed significantly increased number of Aβ labelled cells in layers III and V of the cortex after hypercholesterolemia and hypertension, which may be due to decreased proteasome activity and/or increased β- or γ-secretase activity. Knowledge of altered gene networks during hypercholesterolemia and/or hypertension could inform our understanding of the link between these conditions and cognitive deficits in vascular dementia or Alzheimer's disease.

1. Introduction

Metabolic syndrome which is related to the presence of several conditions that are related to central obesity, such as aberrant glucose metabolism, dyslipidemia and hypertension, is associated with increased risk of type 2 diabetes mellitus and coronary artery disease (Panza et al., 2008). In addition, many studies have reported the association of metabolic syndrome with increased risk of age-related cognitive deficits (Ho et al., 2008), vascular dementia (Solfrizzi et al., 2010) and Alzheimer's disease (AD) (Rios et al., 2014; Watts et al., 2013). Increased plasma or serum cholesterol levels (Jarvik et al., 1995; Kuo et al., 1998; Pappolla et al., 2003), increased low-density lipoprotein (LDL) cholesterol (Kuo et al., 1998; Lesser et al., 2001), or decreased high-density lipoprotein (HDL) cholesterol (Bonarek et al., 2000; McGrowder et al., 2011; Merched et al., 2000; van Exel et al., 2002) have been associated with increased susceptibility to AD. In contrast, elderly individuals with elevated HDL cholesterol have reduced risk of AD (Reitz et al., 2010). Statins that reduce cholesterol biosynthesis, have been reported to reduce the risk of late-onset AD (Haag et al., 2009) and help in prevention of AD (Silva et al., 2013).

Induction of hypercholesterolemia in rabbits results in increased deposition of cerebral β-amyloid (Aβ) (Sparks et al., 1994). Likewise,

* Corresponding author. Department of Anatomy, National University of Singapore, 119260, Singapore.
E-mail address: wei_yi_ong@nuhs.edu.sg (W.-Y. Ong).

http://dx.doi.org/10.1016/j.neuint.2016.11.010

induction of hypercholesterolemia in the amyloid-β protein precursor (APP)/presenilin-1 (PS-1) double-transgenic mouse model accelerates Aβ pathology (Refolo et al., 2000). AD mice fed with a high cholesterol diet show greater impairment of spatial learning than those on a normal diet (Li et al., 2003). This deficit is associated with an AD-like pathology, such as increased cortical Aβ and phosphorylated tau (Granholm et al., 2008; Ullrich et al., 2010). Cholesterol lowering-drugs decrease cholesterol levels not only in the plasma but also the brain, after high fat/cholesterol diet and reduce Aβ formation (Refolo et al., 2001).

Besides hypercholesterolemia, hypertension is a risk factor for vascular dementia (Gorelick et al., 2011) and AD (Faraco and Iadecola, 2013; Iadecola, 2013). Increased amyloid plaques and neurofibrillary tangles are found in the brains of patients with hypertension (Rodrigue et al., 2013), and elevated midlife diastolic blood pressure is associated with amyloid angiopathy and risk of AD (Shah et al., 2012). Aβ-immunoreactivity and Aβ-fragments are found in areas of blood-brain-barrier (BBB) damage, in mouse models of hypertension (Carnevale and Lembo, 2011; Gentile et al., 2009).

Although the above studies have identified a link between hypercholesterolemia and/or hypertension and cognitive deficits, till date, comprehensive gene expression analyses of the brain under these conditions is lacking. The present study was carried out to elucidate differential gene expression changes in the prefrontal cortex (PFC) of New Zealand white rabbits exposed to hypercholesterolemia and/or hypertension, with a view of identifying genes and networks at risk.

2. Materials and methods

2.1. Animals

The cholesterol-fed New Zealand white rabbit is the gold standard animal model in atherosclerosis studies (Yanni, 2004). It is often used as a non-transgenic animal model to study the detrimental effect of hypercholesterolemia on the brain (Marwarha et al., 2010), as it demonstrates several of the pathological markers of AD (Deci et al., 2012; Ghribi et al., 2006; Sparks et al., 1994, 2000; Sparks and Schreurs, 2003). We decided to use rabbits rather than rodents in this study, as we wanted to compare the effects of hypercholesterolemia and/hypertension in the same species, and also wanted to avoid a genetic model of hypercholesterolemia, which might affect the interpretation of global gene expression results. It is difficult to induce hypercholesterolemia in rodents (Russell and Proctor, 2006), although hypertension can be produced (Doggrell and Brown, 1998). There is also greater similarity between rabbits and humans, both genetically (Graur et al., 1996) and in their Aβ sequence (Johnstone et al., 1991).

Male rabbits of approximately 8 weeks old (young adults) and weighing 2.0–2.5 kg each at the start of the experiments were used. Three sets of analyses were carried out: i) to determine gene expression changes in the PFC after hypercholesterolemia plus sham operation, ii) to determine gene expression changes in the PFC after hypercholesterolemia plus hypertension, and iii) to determine gene expression changes in the PFC after hypertension. The Goldblatt 2-Kidney 1-Clip (2K1C) method was used to induce hypertension. Analyses were carried out on: a) 6 rabbits on a high cholesterol diet plus sham operation. b) 6 rabbits on a high cholesterol diet plus the 2K1C method to induce hypertension. c) 6 rabbits with 2K1C-induced hypertension and fed with normal diet. d) 6 sham-operated controls on a normal diet (controls). Rabbits were sacrificed at the end of 12 weeks after surgery or sham operation.

The 2K1C procedure to induce hypertension was performed as previously described (Akabane et al., 1985; Ong et al., 2013). Animals in the 'hypercholesterolemia plus sham/hypertension' groups were allowed to recover for 1 week after surgery before proceeding with dietary treatment containing cholesterol. The diet for these groups of rabbit was GPR diet +1% cholesterol (Glen Forrest Stockfeeders, Australia). 'Sham operated control' rabbits were fed with GPR diet without cholesterol. All procedures including animals were approved by the Institutional Animal Care and Use Committee of the National University of Singapore, and carried out in accordance with guidelines of the National Advisory Committee for Laboratory Animal Research.

2.2. Measurement of body weight, mean arterial pressure and serum total cholesterol

Rabbits were anaesthetized by intramuscular injection of ketamine/xylazine cocktail, and mean arterial pressure measurements and blood collection carried out as previously described (Ong et al., 2013).

2.3. Tissue harvesting and RNA extraction

The different groups of rabbits were sacrificed and tissues harvested as previously described (Ong et al., 2013).

2.4. DNA microarray analysis

Ten μL of total RNA from the PFC of four rabbits from each group were submitted to Genomax Technologies, Singapore. RNA quality was validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). cRNA was generated, labelled using the one-cycle target labeling method, and hybridized to the 1-colour Agilent Rabbit Microarray (G2519F-020908; Agilent Technologies), according to the manufacturer's protocol. Data was collected and exported to GeneSpring v11 software for analysis, using a parametric test based on the cross gene error model (Agilent Technologies). Differentially expressed genes (DEGs) are those that show significantly increased or decreased expression compared with 'sham operated controls' using one-way ANOVA with Tukey HSD post-hoc test, and corrected for multiple comparisons using Benjamini Hochberg FDR ($P < 0.01$). DEGs from the 'hypercholesterolemia plus sham' operated group vs. 'sham operated controls' were also compared between the PFC and MCA, using unpaired T-test and Benjamini Hochberg FDR ($P < 0.01$). Data for the MCA was based on our previous study (Ong et al., 2013), which used the same rabbits as this study. To minimize false positives, only DEGs with greater than 4-fold change (or in the case of common genes between two data sets greater than 4-fold change in at least one data set) with $P < 0.01$ were presented, and used for Ingenuity Pathway Analysis (IPA) analyses, below.

2.5. Network analyses

DEGs in the PFC were further analyzed by IPA software (Ingenuity® Systems, www.ingenuity.com), to elucidate possible functional interactions between genes. Identifiers and corresponding expression values of up- or down-regulated DEGs with more than 4-fold change were uploaded into the IPA software. Each identifier mapped to its corresponding object, and was overlaid onto a global molecular network in the Ingenuity® Knowledge Base. Focus Genes (Network Eligible genes) are defined as DEGs that have at least one other molecule that interacts with it, in the Knowledge Base.