Short Communications

Role of cytochrome P450 for vitamin D metabolisms in patients with neurodegenerative disorders

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ABSTRACT

Introduction: We previously reported lower serum 25-hydroxyvitamin D concentrations in patients with Alzheimer's disease (AD), Parkinson's disease (PD) and Multiple system atrophy (MSA) compared to healthy controls (HC), whereas 1,25-di-hydroxyvitamin D levels were solely lower in MSA patients. We investigate serum concentrations of P450 involved in Vitamin D(VD) hydroxylation to clarify the responsible hydroxylase for the low serum concentrations of VD metabolites.

Methods: A total of 79 individuals were enrolled including 20 HC, 20 AD, 19 PD and 20 MSA patients. The serum concentrations of P450 involved in VD hydroxylation were assayed by ELISA. The data were analyzed by the nonparametric Kruskal-Wallis test between groups.

Results: Though CYP2R1 and CYP27A1 mediate 25-hydroxylation for VD, CYP2R1 is the main hydroxylase, and CYP27A1 is also involved in VD synthesis. CYP2R1 concentrations showed no differences among groups, while lower CYP27A1 concentrations were found in PD ($p < 0.05$) and MSA ($p < 0.005$) compared to HC and differences between AD and MSA ($p < 0.05$), however no differences between PD and MSA. CYP27B1 is the main 1α-hydroxylase for 25-hydroxyvitamin D and showed differences between HC and PD ($p < 0.05$), between HC and MSA ($p < 0.005$) and between PD and MSA ($p = 0.055$). CYP24A1, which inactivate 1,25-di-hydroxyvitamin D, showed no differences among groups.

Conclusions: CYP27A1 might affect VD synthesis and cause low 25-hydroxyvitamin D levels in AD, PD and MSA patients. Low 1,25-di-hydroxyvitamin D levels in MSA patients might be caused by impaired feedback mediated by CYP27B1.

1. Introduction

Recently, many reports suggested the pleiotropic actions of Vitamin D (VD) in brain functions including neuronal development, neuro-plasticity and neuro-protections and the deficiency is a risk factor of neurodegenerative disease with dementia [1].

We previously reported lower serum concentrations of 25-hydroxyvitamin D (25(OH)D) in patients with Alzheimer's disease (AD), Parkinson's disease (PD) and Multiple system atrophy (MSA) compared to healthy controls (HC), whereas 1,25-di-hydroxyvitamin D (1,25(OH)$_2$D) levels were solely lower in MSA patients and suggested the usefulness of

serum 1,25(OH)$_2$D levels for the differential diagnosis [2,3]. The cause of lower VD metabolites level in these patients remains unknown.

In the present study, we aim to study the common cause of lower 25(OH)D levels in patients with AD, PD and MSA, and the specific cause of lower 1,25(OH)$_2$D levels in patients with MSA. We focus on serum concentrations of hydroxylases involved in VD metabolisms.

The synthesis of VD in the skin is the most important source of VD. Ultraviolet exposure is the initial step to produce Pre-VD from 7-dehydrocholesterol in the skin and followed by a temperature-sensitive rearrangement of three double bonds to form VD. VD is sequentially activated by 25- and 1α-hydroxylase to form 25(OH)D in the liver and

1,25(OH)$_2$D in the kidney, respectively and followed with inactivation by 24-hydroxylase. The serum concentration of 25(OH)D is a biomarker of VD status. Serum concentration of 1,25(OH)$_2$D indicates 1α-hydroxylase activity in the kidney and feedback regulation of VD metabolisms by 1,25(OH)$_2$D is reported [4]. Decreased serum 25(OH)D concentrations upregulate 1α-hydroxylase activity and increase serum1,25(OH)$_2$D levels, otherwise extreme deficiency of 25(OH)D or Sarcoidosis.

The biological actions of 1,25(OH)$_2$D are mediated by VD receptor (VDR), which belong to the steroid receptor and is expressed in many organs including kidney, small intestine, parathyroid gland, bone tissue, skin and brain. The binding of VD to VDR induce signal transduction to cells and the affinity of 1,25(OH)$_2$D to VDR are 500 folds stronger than that of 25(OH)D, however serum concentrations of 25(OH)D are 1000 folds higher than that of 1,25(OH)$_2$D.

The main enzyme involved in 25-hydroxylation for VD are CYP2R1 and CYP27A1.

The CYP27B1 in the kidney is the main 1α-hydroxylase for 25(OH)D, though CYP27A1 have some activity of 1α-hydroxylase. CYP24A1 is involved in degradation of VD metabolites, and it is contained in VD target organs such as kidney and mucosa of small intestine. The binding of 1,25(OH)$_2$D to VDR induce increase of CYP24A1 concentrations, which inactivate 1,25(OH)$_2$D by hydroxylation at a position of 24, inhibiting the affinity of 1,25(OH)$_2$D to VDR and regulate the serum 1,25(OH)$_2$D levels. These hydroxylases belong to cytochrome P450 family and similarity of amino acids sequences between CYP 27A1 and CYP 27B1 is about 40 %. The CYP 27A1, CYP27B1 and CYP24A1 are in mitochondria (mitochondria type), while CYP2R1 is in microsome (microsome type). We assayed serum concentrations of cytochrome P450 in these patients with neurodegenerative disorders and healthy control by ELISA and discuss the responsible P450 as a factor for the lower concentrations of VD metabolites. To our knowledge, this is the first report for the serum concentrations of VD hydroxylases in neurodegenerative disorders.

2. Materials & methods

This study was approved by the institutional ethics committee at Faculty of Medicine, Fukuoka University, Japan (BIR No. 2018M030). Diagnosis was performed according to the clinical diagnostic criteria for AD, PD and MSA respectively [5]. All patients and healthy controls enrolled in this study were free from hepatic and renal dysfunction and they don't take VD supplements. The serum concentrations of cytochrome P450 (CYP27A1, CYP2R1, CYP27B1, CYP24A1) were analyzed by ELISA kits such as CYP27A1 Kit (Cloud-Clone Corp. TX, USA), CYP2R1 Kit (CUSABIO TECHNOLOGY LLC. TX, USA), CYP27B1 Kit (Aviva Systems Biology Corp. CA, USA) and CYP24A1 Kit (CUSABIO TECHNOLOGY LLC. TX, USA) using ELISA plate reader; Infinite 200 PRO (Tecan, Hombrechtikon, Switzerland) at the wavelength of 450 nm.

The data were analyzed by the nonparametric Kruskal-Wallis test among AD, PD, MSA and HC. Statistical significance was set at $p < 0.05$. Moreover, we conducted the linear regression analysis to evaluate the extent of influence of the independent predictors (age, sex) in all patients including AD, PD and MSA, and the predictors (Hoehn and Yarn scale, disease duration) in PD, and the predictors (Unified MSA rating scale IV, disease duration) in MSA on the dependent variables (CYP 27A1, CYP27B1, CYP2R1 and CYP24A1). The following parameters were calculated: unstandardized (USC) and standardized (SC) coefficients *B* and beta(β) respectively, R-squared (R^2). The predictors were tested with univariate logistic regression analyses to assess the contribution of each predictor. Statistical analyses were conducted using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1 shows the demographic characteristics of the participants. A total of 79 individuals were enrolled in this study including 20 AD

Table 1
Demographic characteristics, serum concentrations cytochrome P450 in HC, AD, PD and MSA patients.

Disease	HC	AD	PD	MSA
Total number (n)	n = 20	n = 20	n = 19	n = 20
Women	n = 9	n = 10	n = 10	n = 11
Men	n = 11	n = 10	n = 9	n = 9
Age (year)	75. ± 6.57	79.3 ± 6.38	69.8 ± 4.64	61.2 ± 8.36
[range]	[63–87]	[65–89]	[61–77]	[49–80]
Disease duration (month)	–	ND	93.9 ± 60.9	59.5 ± 44.6
Disease severity	–	ND		
H&Y			2.47 ± 0.60	
UMSARS IV				3.0 ± 1.15
25(OH)D (ng/mL)	24.3 ± 6.72	17.6 ± 6.23	14.74 ± 4.65	10.51 ± 3.56
1,25(OH)$_2$D (pg/mL)	54. ± 11.17	51.5 ± 17.88	63.03 ± 14.61	39.5 ± 13.50
P (mg/dL)	ND	ND	3.32 ± 0.41	3.49 ± 0.52
PTH (pg/mL)	ND	ND	47.32 ± 21.68	39.33 ± 11.68
TC (mg/dL)	206 ± 41	197 ± 23	187 ± 30	186 ± 42
CYP27A1 (ng/mL)	0.22 ± 0.21	0.14 ± 0.19	0.08 ± 0.07	0.05 ± 0.09
CYP27B1 (ng/mL)	0.24 ± 0.21	0.17 ± 0.09	0.12 ± 0.08	0.08 ± 0.10
CYP2R1 (pg/mL)	37.15 ± 17.76	39.52 ± 21.0	31.09 ± 13.33	28.29 ± 16.48
CYP24A1 (pg/mL)	35.47 ± 18.01	39.2 ± 22.61	31.73 ± 15.16	28.74 ± 12.41

Data express mean ± SD. PTH; parathyroid hormone, P; phosphate, TC; Total cholesterol.
ND; not done, H&Y; Hoehn & Yahr, UMSARS IV; Unified multiple system atrophy rating scale IV, HC; Healthy control, AD; Alzheimer's disease, PD; Parkinson's disease, MSA; multiple systemic atrophy.

patients,19 PD patients, 20 MSA patients and 20 HC, and all groups were gender-balanced. As MSA is a rapid progressive disease and lifespan of the patients are shorter than that of other neurodegenerative disorders, the age of enrolled MSA patients are younger than that of other neurodegenerative disorders.

The serum concentrations of CYP27A1 were 0.22 ± 0.21 ng/ml in HC, 0.14 ± 0.19 ng/ml in AD, 0.08 ± 0.07 ng/ml in PD and 0.05 ± 0.09 ng/ml in MSA. We found significant differences in the serum concentration of CYP27A1 between HC and PD ($p < 0.05$) as well as HC and MSA ($p < 0.005$) and between AD and MSA ($p < 0.05$) (Fig. 1-a). The serum concentrations of CYP2R1 were 37.15 ± 17.76 pg/ml in HC, 39.52 ± 21.02 pg/ml in AD, 31.09 ± 13.33 pg/ml in PD and 28.29 ± 16.48 pg/ml in MSA and found no significant differences among groups (Fig. 1-d). The serum concentrations of CYP27B1 were 0.24 ± 0.21 ng/ml in HC, 0.17 ± 0.09 ng/ml in AD, 0.12 ± 0.08 ng/ml in PD and 0.08 ± 0.10 ng/ml in MSA and showed significant differences between HC and MSA ($p < 0.005$) and between HC and PD ($p < 0.05$), between PD and MSA ($p = 0.055$), (Fig. 1-b). The serum concentrations of CYP24A1 were 35.47 ± 18.01 pg/ml in HC, 39.2 ± 22.61 pg/ml in AD, 31.73 ± 15.16 pg/ml in PD and 28.74 ± 12.41 pg/ml in MSA and showed no significant differences among groups (Fig. 1-c). The serum concentrations of parathyroid hormone (PTH) were 47.32 ± 21.68 pg/ml in PD and 39.33 ± 11.68 pg/ml in MSA, and that of phosphate were 3.32 ± 0.41 mg/dL in PD and 3.49 ± 0.52 mg/dL in MSA. They were within normal ranges and no significant differences in serum concentrations of PTH and phosphate between PD and MSA. The serum concentrations of total cholesterol were 206 ± 41 mg/dL in HC, 197 ± 23 mg/dL in AD,187 ± 30 mg/dL in PD and 186 ± 42 mg/dL in MSA and found no significant differences among groups (Table 1).

The linear regression analysis (supplementary Table) showed only in MSA patients, the disease duration exerted significant effect on CYP27A1 concentrations ($p < 0.0001$). The USC coefficient $B = +0.002$ indicates this unit increase of CYP27A1 for every month-long disease
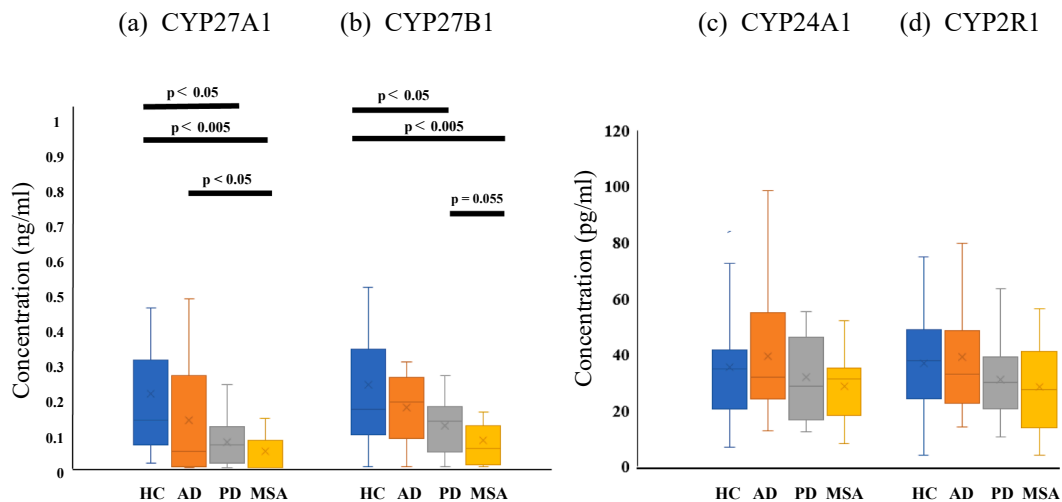
(a) CYP27A1   (b) CYP27B1   (c) CYP24A1   (d) CYP2R1

Fig. 1. Serum concentrations of CYP27A1, CYP27B1, CYP2RI and CYP24A1 in HC, AD, PD and MSA patients.

duration; SC β = +0.737 indicates this SC increase of CYP27A1 for every SC of the disease duration which appears to share positive 54.3 % (R^2 = 0.543) of the variation in CYP27A1.

4. Discussion

The CYP2R1 (L99P) mutant cause rickets and showed selective 25 (OH)D deficiency, however serum $1,25(OH)_2D$ levels were within normal range in these patients and the results suggest other active form of VD than $1,25(OH)_2D$ [6]. Disrupted CYP27A1 gene in mouse showed reduced synthesis of bile acid, however the serum 25(OH)D level was not affected [7]. The patients with cerebrotendinous xanthomatosis, having the mutated CPY27A1 gene showed accumulation of cholestanol and no decrease of serum 25(OH)D level [8]. These findings suggest that CYP27A1 is the physiologically main 25-hydroxylase for VD and CYP27A1 is also involved in VD synthesis before 25 hydroxylation. Our data showed no significant differences in CYP2R1 among groups (Fig. 1-d), while CYP27A1 showed significant differences in PD and MSA compared to HC, and MSA showed significant differences to AD (Fig. 1-a). We suggest that CYP27A1 might rather affect VD synthesis than 25-hydroxylation for VD. Though we found no significant differences in serum concentrations of total cholesterol among groups, many reports suggested low level of serum cholesterol in PD patients [9].

The linear regression analysis showed significant correlation only between CYP27A1 concentrations and disease durations in MSA patients, however other factors such as age, sex and disease severity showed no correlation between CYP27A1(supplementary Table). The prognosis of MSA is poor and disease duration reflect their lifespan. Our data suggest that serum CYP27A1 levels might be a biomarker for the prognosis in MSA patients.

Lower levels of serum CYP27A1 are common to patients with PD and MSA, and lower level of serum CYP27B1 is specific for patients with MSA (Fig. 1-a,b). These profile of the CYPs match to the serum levels of VD metabolites in our previous study [3].

VD attenuate dopamine dysmetabolism in 6-hydroxydopamine injected mice [10] and lower VD levels caused by lower CYP27A1 in PD patients might affect the dopamine metabolisms. The common pathological feature in PD and MSA brain is aggregated α-synuclein and VD inhibits the early aggregation of α-synuclein [11]. Our results suggest that lower VD levels mediated by lower CYP27A1 in PD and MSA patients might affect early aggregation of α-synuclein.

Though, the serum 25(OH)D level in PD patients were as low as that of MSA patients, CYP27B1 levels in PD patients were higher than that of MSA patients (p = 0.05) (Fig. 1-b) and recovered $1,25(OH)_2D$ levels in PD patients[3]. Many regulatory factors for $1,25(OH)_2D$ synthesis have been suggested. Parathyroid hormone (PTH), lower phosphate concentration and insulin-like growth factor-1 are stimulating factors, and higher phosphate concentration and fibroblast growth factor-23 are inhibiting factors for 1α-hydroxylase activity [12]. Our data showed that serum concentrations of PTH and phosphate were within normal ranges in both PD and MSA patients and no significant differences between PD and MSA (Table 1). Feedback regulation of VD by $1,25(OH)_2D$ itself is another control mechanism for VD metabolisms [4]. We speculate that MSA patient could not upregulate CYP27B1 in response to lower $1,25(OH)_2D$, however PD patients could upregulate CYP27B1. The CYP24A1 catalyzes both of 25(OH)D and $1,25(OH)_2D$ as substrates into 24-hydroxylated products. The C24-C25 bond cleavage by CYP24A1 abolish the binding affinity of $1,25(OH)_2D$ to the VDR and inactivate $1,25(OH)_2D$. CYP24A1 showed no significant differences among groups and have no contribution to the low serum concentrations of $1,25(OH)_2D$ in MSA patients. Our data suggest that the CYP27A1 might affect VD synthesis before 25-hydroxylation and cause low serum concentrations of 25(OH)D in patients with AD, PD and MSA. Low serum $1,25(OH)_2D$ levels in MSA patients might be caused by impaired feedback mediated by CYP27B1.

Patients with neurodegenerative disease spend most of time indoor and insufficient exposure to sunlight might be the main factor for lower levels of VD metabolites, however we suggest low concentrations of CYP 27A1 and CYP 27B1 as another risk factors for low concentrations of VD metabolites in these patients. Administration of $1,25(OH)_2D$ might be useful for therapeutics of MSA patients.

Authors' contributions

Kakimoto A: Data analysis, interpretation and manuscript writing.
Suenaga M: statistical analysis.
Ogura H, Mishima T, Ouma S and Fujioka S: execution of the project.
Matsunaga Y: Conception of the article, design of the study.
Tsuboi Y: project organization, draft of the article.

Ethics

The work described has been carried out in accordance with The Code of Ethics of the world Medical Association (Declaration of Helsinki) for experiments involving human.

All study participants provided informed consent, and the appropriate ethics review board by the institutional ethics committee at Faculty of Medicine, Fukuoka University approved the study design (BIR No. 2018M030).

Submission declaration and verification

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. The publication is approved by all authors.

After acceptance of the manuscript, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.prdoa.2022.100162.

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