Longer Telomere Length of T lymphocytes in Patients with Early and Chronic Psychosis

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Objective: To investigate pathological conditions that act as sources of pro-inflammatory cytokines and cytotoxic substances to examine telomere length (TL) in patients with either early (duration of illness [DI] ≤5 years) or chronic (DI >5 years) psychosis using T lymphocytes.
Methods: Based on these factors and the important role that T lymphocytes play in inflammation, the present study measured the TL of T lymphocytes in patients with either early or chronic psychosis. Additionally, smoking, metabolic syndrome, depression, and cognitive functioning were assessed to control for confounding effects.
Results: TL was significantly longer in patients with early and chronic psychosis than in healthy control subjects and, moreover, the significance of these findings remained after controlling for age, smoking, metabolic syndrome, DI, chlorpromazine-equivalent dose, and cognitive functioning (F=9.57, degree of freedom=2, $p<0.001$). Additionally, the DI, chlorpromazine-equivalent doses, and the five-factor scores of the Positive and Negative Syndrome Scale were not significantly correlated with the TL of T lymphocytes in either all patients or each psychosis group.
Conclusion: Possible mechanisms underlying the effects of antipsychotic medications on telomerase are discussed in the present study, but further studies measuring both telomerase activity and TL using a prospective design will be required.

KEY WORDS: Antipsychotic agents; Telomere length; Telomerase; T lymphocytes; Psychosis; Quantitative Real-time PCR.

INTRODUCTION

Telomeres are nucleoprotein complexes composed of tandem repeat DNA sequences $(TTAGGG)_n$ that form protective caps at the ends of eukaryotic chromosomes. In somatic cells, telomeres progressively shorten with each cell division, which is due to the incomplete replication of linear chromosomes by DNA polymerases. Telomerase is a cellular ribonucleoprotein reverse transcriptase that extends telomeres and, in turn, protects them and maintains chromosomal integrity.[1] Because the expression of telomerase in most somatic cells is insufficient to completely restore the length of telomeres, these nucleoproteins progressively shorten with each cell division. Maintaining the length of telomeres extends the lifetime of cells[1]; thus, telomere length (TL) reflects the proliferative history of cells and serves as a mitotic clock of physical aging in many organisms.[1-3]

For example, the reduction of TL in peripheral blood leukocytes is accelerated by oxidative/metabolic stress and is associated with illnesses related to aging and degenerative phenotypes, such as dementia,[4] cardiovascular disease, and diabetes. In terms of psychological factors, current life stress[5] and personality traits, such as pessimism[6] and hostility,[7] are associated with shorter leukocyte TL. Furthermore, the acceleration of telomere shortening occurs in patients with mood disorders,[8] generalized anxiety disorder, post-traumatic stress disorder,[9] and schizophrenia.[10]

The psychotic symptoms that are commonly associated with schizophrenia are related to the overproduction of reactive oxygen species (ROS) and impaired oxidative defenses.[11] Patients with schizophrenia often exhibit a variety of physical problems, including obesity, metabolic syndrome, diabetes mellitus, cardiovascular disease, and unhealthy lifestyles, which are all associated with reduced

TL.[12] Although many studies have investigated TL in patients with schizophrenia, the link between telomeres and schizophrenia remains elusive. Many studies have reported that psychotic patients exhibit shorter TL compared with healthy controls,[10,13-15] whereas others have found no difference in this regard[16-18] or longer TL.[19] These discrepant findings may be due to the use of different study designs with participants of various ages and genders, different durations of illness (DI), and medication statuses and/or the use of various specimen analyses.

All these studies, except that conducted by,[16] collected peripheral blood mononuclear cells (PBMCs) to assess these issues. Given that B lymphocytes exhibit the longest mean TL and CD8＋CD28－T cells have the shortest mean TL,[20] it was hypothesized that measuring TL in a specific subset of lymphocytes would produce more consistent results. As a result, the present study chose to focus on T lymphocytes because activated T-cells and, to a lesser extent naive/resting T-cells, regularly transmigrate into the central nervous system via the blood-brain barrier under physiological conditions and act as ROS.[21] Thus, the aim of the present study was to investigate pathological conditions[22] that act as sources of pro-inflammatory cytokines and cytotoxic substances to examine TL in patients with either early (DI ≤5 years) or chronic (DI >5 years) psychosis using T lymphocytes.

METHODS

Subjects

Patients were recruited from the outpatient clinic of the Department of Psychiatry at Chonbuk National University Hospital between July 2015 and December 2015. The inclusion criteria were as follows: 1) diagnosis of a schizophrenia spectrum disorder (schizophrenia, schizoaffective disorder, schizophreniform disorder, psychotic disorder not otherwise specified [NOS], or brief psychotic disorder [BPD]), attenuated psychosis syndrome (APS), or a delusional disorder according to the criteria of the Structural Clinical Interview from the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (SCID-IV)[23,24]; 2) aged between 19 and 65 years; and 3) the ability to comprehend the procedure and aims of the present study. Criteria from the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) were applied to diagnose APS.

The exclusion criteria were as follows: 1) diagnosis of mental retardation (intelligence quotient ＜70); 2) history

of head trauma; 3) serious neurological disorder (epilepsy, stroke, Parkinson's disease, and/or dementia); and/or 4) an acute, unstable, and significant medical illness. In total, 35 healthy individuals were recruited for the control group via advertisements; these participants were age- and sex-matched to the patients with early psychosis and interviewed using the screening module of the SCID-IV, non-patient edition.[24,25] Control subjects were excluded from the present study based on the following: a current or previous diagnosis of a psychiatric or neurological disorder, alcohol or drug abuse or dependence (except for nicotine), and/or another significant medical condition. All participants provided written informed consent in accordance with a protocol approved by the Ethics Committee of the Chonbuk National University Hospital (approval number 2015-04-027-008).

Measures

Data on sociodemographic (age, sex, education, marital status, occupation, and smoking) and clinical (DI, medication, and metabolic syndrome) variables were obtained. The severity of psychiatric symptoms was measured using the Positive and Negative Syndrome Scale (PANSS)[26]; depressive features were evaluated using the Calgary Depression Scale for Schizophrenia (CDSS)[27]; and cognitive functioning was assessed with the Clinical Global Impression Scale for Cognitive Symptoms of Schizophrenia (CGI-C).[28] Metabolic syndrome was defined according to the criteria of the third report of the National Cholesterol Education Program, Adult Treatment Panel (NCEP ATP III)[29] in which the World Health Organization guidelines for abdominal obesity (2000) were applied. Daily doses of antipsychotic medications were converted into chlorpromazine-equivalent doses using an equivalency table provided by Hales.[30] For blonanserin, 4 mg was considered to be equivalent to 1 mg of risperidone based on previous studies.[31,32] Similarly, the ratio of paliperidone extended release (ER) to risperidone was determined to be 2, because the effective dose 50 values of striatal dopamine D2 receptor occupancy for paliperidone ER and risperidone are 2.38 mg/day[33] and 1.2 mg/day,[34] respectively.

Isolation of T Lymphocytes

A blood sample from the fasting ulnar vein (10 ml) was drawn from each individual and collected in vacutainer tubes containing preservative-free heparin. Next, PBMCs were isolated using standard density centrifugation (2,500 rpm for 30 min at 25°C without a break) with Ficoll-Paque PLUS medium (d=1.077; Amersham Pharmacia Biotech,

Uppsala, Sweden). Red blood cell lysis buffer (BioLegend, San Diego, CA, USA) was used to remove residual red blood cells, and the platelets were then removed by centrifugation (1,000 rpm for 15 min at room temperature). Finally, the purification of the T lymphocytes was performed with a T cell enrichment column (R&D Systems, Minneapolis, MN, USA), and the lymphocytes were kept at -70°C until further analysis. According to a flow cytometry analysis (BD Biosciences, San Jose, CA, USA), the purity of the T lymphocytes was $>92\%$ after staining with monoclonal antibodies for human CD3 antigens PE (BD Biosciences). The entire procedure was completed within 4 hours of drawing the blood samples.

Determination of TL Using Quantitative Real-time Polymerase Chain Reaction Analyses

The measurement of relative TL was conducted using a quantitative real-time polymerase chain reaction (PCR) assay adapted from the original method.[35] DNA was extracted from the T lymphocytes with a QIAamp DNA Mini kit (Qiagen, Hilden, Germany), and the quantity and quality were determined using a Colibri Microvolume Spectrophotometer 4 (Titertek-Berthold, Pforzheim, Germany); a density ratio of 260/280 nm >1.7 was considered to be acceptable. DNA samples (5 ng) were processed in triplicate for both the telomere and the single-copy hemoglobin-b (Hgb) gene with the Applied Biosystems 7900HT Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA). The reaction system contained 5 ng of DNA, 5 μl of 1× Power SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), and various primers (Bioneer Inc., Seoul, Korea).

The primers used in the present study were as follows: the telomere PCR primers were 100 nM of Tel1 (5' CGG TTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGG TT-3') and 900 nM of Tel 2 (5'-GGCTTGCCTT ACCCTTACCCTTACCCTTACCCTTACCCT-3'), and the Hgb PCR primers were 300 nM of Hgb1 (5'-GCT TCTGACACAACTGTGTTC ACTAGC-3') and 700 nM of Hgb2 (5'-CACCAACTTC ATCCACGTTCACC-3'). The reaction condition for the telomere was 50°C for 2 min, 95°C for 10 min, 95°C for 15 sec, 56°C for 30 sec, and 72°C for 30 sec (40 cycles). The reaction condition for Hgb was 50°C for 2 min, 95°C for 10 min, 95°C for 15 sec, 58°C for 20 sec, and 72°C for 20 sec (40 cycles). No-template control and dissociation curve analyses were performed to indicate specificity. A dilution series of a pooled of DNA (33.33, 5.556, 0.926, 0.154, and 0.026 ng) for both telomere and Hgb was included in each plate to create

reaction-specific standard curves and R^2 values; both the telomere and Hgb reaction values were >0.99. Four independent samples were included in each plate to control the variation between different plates, and the cycle threshold (Ct) mean values of the independent samples in each plate were used as calibrators. The relative TL was calculated using the comparative $2^{-\Delta\Delta Ct}$ method: $\Delta Ct= Ct_{Tel}-Ct_{Hgb}$, $\Delta\Delta Ct=\Delta Ct_{sample}-\Delta Ct_{calibrator}$. The intra-assay analysis in the present study excluded the samples from the triplicates with a standard deviation >0.5.[36] The coefficients of variation (CVs) for the triplicate samples were 0.78% for the telomere reaction and 0.40% for the Hgb reaction, which indicated low measurement error.[37] For the inter-assay analysis, the CVs for the independent samples between the different plates were 1.18% for the telomere reaction and 0.45% for the Hgb reaction.

Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics ver. 20.0 (IBM Co., Armonk, NY, USA), and p values <0.05 were considered to indicate statistical significance. The Shapiro-Wilk test was employed to assess the normality of the TL data; t tests and analysis of variance (ANOVA) and chi-square tests were carried out to assess the continuous and categorical variables, respectively. Bonferroni corrections were made for multiple comparisons, and age, number of cigarettes per day, metabolic syndrome, DI, daily doses of antipsychotics, and cognitive function were included as covariates to control for confounding effects on TL. Pearson correlation tests were conducted to analyze relationships between TL and age or PANSS scores.

RESULTS

The demographic and clinical characteristics of participants are presented in Table 1. The diagnoses for the early psychosis group were as follows; schizophrenia (n=24), schizophreniform disorder (n=10), psychotic disorder NOS (n=5), BPD (n=1), and APS (n=3). The diagnoses for the chronic psychosis group were as follows; schizophrenia (n=75), schizoaffective disorder (n=3), schizophreniform disorder (n=1), psychotic disorder NOS (n=3), and delusional disorder (n=1). There were significant differences between the two patient groups and the control group in terms of age, smoking, occupation, marriage, and chlorpromazine-equivalent dose.

The *post-hoc* results revealed that the mean age of the patients with chronic psychosis was significantly older

Table 1. Demographic and clinical characteristics of participants

Variable	Control (n=35)	Early psychosis (n=43)	Chronic psychosis (n=83)	p value
Age (yr)	32.06±8.64*	31.63±11.10*	42.10±9.72	<0.001
Sex, male/female	16/19	22/21	47/36	0.538
Education				
Elementary school	0	0	5 (6.0)	0.232
High school	13 (37.1)	20 (46.5)	32 (38.6)	
University or higher	22 (62.9)	23 (53.5)	46 (55.4)	
Number of cigarettes/day	0	3.88±7.53[†]	3.28±7.04[†]	0.017
Occupation				
Student	10 (28.6)	9 (20.9)	11 (13.3)	<0.001[†]
Unemployed	12 (34.3)	24 (55.8)	62 (74.7)	
Employed	13 (37.1)	10 (23.3)	10 (12.0)	
Marital status				
Never married	16 (45.7)	33 (76.8)	50 (60.2)	0.012[†]
Married	19 (54.3)	7 (16.3)	25 (30.1)	
Married but separated, or divorced	0	3 (7.0)	6 (7.2)	
Widowed	0	0	2 (2.4)	
Chlorpromazine equivalent (mg/day)	0*,§	311.50±320.85*	641.76±445.70[†],§	<0.001
Metabolic syndrome	-	7 (16.3)	28 (33.7)	0.039
Disease characteristics				
DI	-	34.49±45.27	172.27±90.40	<0.001
CGI-C	-	1.98±0.94	2.78±1.10	<0.001
CDSS	-	3.35±3.86	2.18±2.96	0.061
Positive PANSS	-	13.12±7.31	12.06±5.45	0.362
Negative PANSS	-	12.61±6.20	13.76±5.18	0.299
General PANSS	-	24.54±8.71	21.92±4.79	0.031
Total PANSS	-	50.26±19.29	47.74±12.44	0.734

Values are presented as mean±standard deviation or number (%).
DI, duration of illness; CGI-C, Clinical Global Impression Scale for Cognitive Symptoms of Schizophrenia; CDSS, Calgary Depression Scale for Schizophrenia; PANSS, Positive and Negative Syndrome Scale.
*Significantly different from chronic psychosis; [†]significantly different from control; [†]significantly different among three groups; [§]significantly different from early psychosis.

($p < 0.001$) than was those of the controls and the patients with early psychosis; there were no significant differences in smoking between patients with early and chronic psychosis; and the chlorpromazine-equivalent dose was significantly higher ($p < 0.001$) in patients with chronic psychosis than in patients with early psychosis. Because metabolic syndrome was not assessed in the control group, comparisons for this variable were made only between the patient groups; the prevalence of metabolic syndrome was higher (p=0.039) in patients with chronic psychosis than in patients with early psychosis. In terms of disease characteristics, patients with chronic psychosis had significantly higher scores on the CGI-C ($p < 0.001$) and significantly lower scores on the general PANSS (p=0.031).

Regarding TL, there were significant differences among the three groups (F=26.74, degree of freedom [df]=2, $p < 0.001$), and the *post-hoc* analyses revealed that the patients with early (1.842±0.752) and chronic (1.763± 0.460) psychosis had significantly longer TL values of the T lymphocytes compared with the control group (1.063± 0.752). These findings remained significant after controlling for age, smoking, metabolic syndrome, DI, chlorpro-
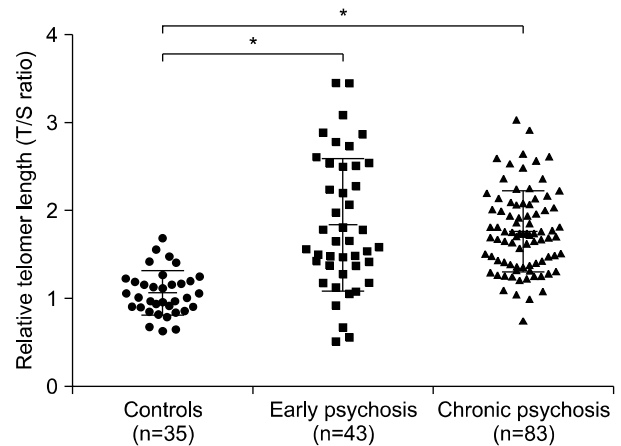

Fig. 1. Relative telomere length among three groups determined by quantitative real-time polymerase chain reaction. Scatter plot graphs illustrate relative individual's relative telomere length (mean and standard deviation).
*Significant differences ($p<0.001$); T/S, telomere to single copy gene.

mazine-equivalent dose, and cognitive functioning (F=9.57, df=2, $p < 0.001$, Fig. 1). No significant correlations were observed between age and the TL of the T lymphocytes in

the overall samples (r=0.103, *p*=0.192), the separate group of controls (r=−0.222, *p*=0.200), or all patients (r=−0.002, *p*=0.982). Additionally, the DI, chlorpromazine-equivalent doses, and the five-factor scores of the PANSS were not significantly correlated with the TL of T lymphocytes in either all patients or each psychosis group.

DISCUSSION

Because high mortality rates, poor physical condition, and vulnerability to stress are associated with schizophrenia and affect TL, the present study measured TL in T lymphocytes. The results showed that the TL values in the early and chronic psychosis groups were significantly longer than those in the control group. These findings remained significant after controlling for covariates such as age, smoking, metabolic syndrome, DI, chlorpromazine-equivalent dose, and cognitive functioning. This was an unexpected finding because most previous studies[10,13-15] found a shorter TL in patients with schizophrenia.

On the other hand, the study conducted by Nieratschker *et al*.,[19] which is the largest-scale study (n=539) in the field of TL research performed to date, found that patients with schizophrenia had longer TL than healthy controls. These authors suggested four alternative explanations; 1) an association with endophenotype (hippocampal volume and/or impaired episodic memory), 2) the effects of psychotropic medications, 3) advanced paternal age, and 4) an association with increased mortality. Wikgren *et al*.[38] reported that healthy apolipoprotein E (APOE) ε3/ε3 individuals with increased TL exhibit reduced hippocampal volumes and that APOE ε4 carriers with longer telomeres show poorer performance during episodic memory tasks.[39] The present study did not objectively measure cognitive functioning or hippocampal volume, and controlling for cognitive function using CGI-C scores did not produce different results. However, it should be noted that the mean CGI-C scores of the patient groups were < 3 (mild level). Moreover, the issues of paternal age and mortality could not be addressed in the present study because the mean age of the chronic psychosis group was 42 years, and information regarding paternal age was not obtained.

The most intriguing interpretations emerging from the present findings involve the association between antipsychotic use and longer TL. Several studies in both animal[40] and human subjects[41,42] indicate that atypical antipsychotics have antioxidant properties. Oxidative stress stimulates the export of telomerase reverse transcriptase (TERT), which is a catalytic protein component of telo-

merase, from the nucleus into the cytosol[43]; this process contributes to decreased telomerase activity and, subsequently, shorter TL. It is possible that the atypical antipsychotics used by the patients in the present study may have blocked this effect and led to the present results: that is, a longer TL. Other suggested mechanisms by which antipsychotics increase TERT expression and telomerase activity include the modulation of intracellular Wnt/β-catenin or PI3K/Akt signaling pathways that is triggered by increases in brain-derived neurotrophic factor (BDNF) expression and 5-hydroxytryptamine (5-HT) modulation.[44,45]

However, the results of several studies contradict these theories. When peripheral blood lymphocytes were obtained from healthy volunteers administered typical and/or atypical antipsychotics within the respective therapeutic ranges normally administered to patients, no effect of telomerase activity was observed for either type of drug.[46] Furthermore, either decreases[14] or no alterations[47] in TL were observed in patients with first-episode/newly diagnosed antipsychotic-naïve schizophrenia. In subanalyses of the present data, no significant associations were found between the chlorpromazine-equivalent doses and DI or TL (data not shown). Thus, it seems essential that future studies measure both telomerase activity and TL within a prospective design. Another mechanism underlying this relationship could be related to the immunosuppressive effects of antipsychotics. Clozapine and haloperidol inhibit the proliferation of human lymphocytes[48]; more importantly, the in vitro stimulation of the T lymphocytes of medicated schizophrenia patients with anti-CD3 produces lower proliferative responses compared with those observed in well-matched controls.[49] Therefore, decreased cellular senescence may have contributed to the longer TL observed in the present study. Regarding the correlation analyses, the lack of an association between age and TL may have been due to the fact that the mean age of the participants was younger than 42 years. Previous studies also found no significant correlations in healthy controls[13,18] or patients with schizophrenia. Similarly, the lack of an association between PANSS scores and TL in the present study may be related to the lower mean PANSS scores in the patient groups.

Given that T lymphocytes are a promising neural candidate for studying psychiatric disorders[50] and all previous studies have measured TL using PBMCs, the strength of the present study is the fact that it is the first to investigate TL in patients with psychosis using T lymphocytes. However, several limitations of this study should be men-

tioned. First, lifestyle factors, such as exercise and eating, affect TL.[51,52] Because low levels of physical activity and poor eating habits are associated with schizophrenia,[53] these factors should be measured and controlled for in future studies. Second, the TL of naïve T cells is longer than that of memory T cells,[54] and changes in the proportion of activated or memory T cells have been reported in patients with schizophrenia.[55,56] Thus, measuring TL in subpopulations of T lymphocytes would produce more accurate and consistent results. Third, the sample size of the early psychosis group in the present study was relatively small.

In conclusion, the T lymphocytes of patients with early and chronic psychosis had longer TL values compared with those of the healthy control group, and this finding remained significant after controlling for various confounding factors. Possible interpretations of the effects of antipsychotics on TL were offered.

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