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ORIGINAL INVESTIGATION

Association between telomere length in the DNA of peripheral blood leukocytes and the propofol dose in anesthesia induction: an observational study

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KEYWORDS
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Abstract
Introduction: Propofol is a widely used anesthetic and its dose is closely related to aging. Telomere length (TL) is a unique heritable trait, and emerging as a biomarker of aging, health and disease. Telomerase RNA component (*TERC*) plays an important role in maintaining TL. We proposed a hypothesis that propofol dose in general anesthesia can be predicted by measuring TL before operation, which greatly reduced the risk of anesthesia, especially the elderly.
Methods: The association between the propofol dose in anesthesia induction and: TL in the DNA of peripheral blood leukocytes; body weight; sex; difference of the Bispectral Index (BIS) before and after anesthesia induction in patients was evaluated by multivariable linear regression analyses. The mutation at the 5'end or 3'end of *TERC* was detected. We recruited 100 patients of elective surgery.
Results: We found that propofol dose in anesthesia induction was clearly correlated significantly with TL (r = 0.78, $p < 0.001$), body weight (r = 0.84, p = 0.004), sex (r = 0.83, p= 0.84, $p = 0.004$), sex (r = 0.83, $p = 0.004$), and difference of BIS before and after anesthesia induction (r = 0.85, $p = 0.029$). By comparing the absolute values of standardized regression coefficients (0.58, 0.21, 0.19, and 0.12) of the four variables, it can be seen that TL contributes the most to the propofol dose in anesthesia induction. However, the mutation at the 5' end or 3' end of *TERC* was not found.

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Conclusions: These findings provide preliminary evidence that the propofol dose in anesthesia induction was clearly correlated with genetically determined TL. TL may be a promising predictor of the propofol dose, which is beneficial to improve the safety of anesthesia and reduce perioperative complications.

Introduction

The elderly constitutes the most important population within the general population with regard to complications and deaths from anesthesia and surgery as the older population grows.[1] Tolerance to intravenous general anesthesia decreases gradually with age in humans.[2] To date, there are no precise criteria for assessing the change in tolerance of the body to anesthetic drugs. Telomeres are specialized protein-bound DNA repetitive sequences at the end of eukaryotic chromosomes. They regulate the replication and proliferation of cells, avoid chromosome fusion during mitosis, and maintain genomic stability.[3] Telomerase RNA component (*TERC*) serves as a template and synthesizes DNA telomere repeats to maintain telomere length (TL). TL is a unique heritable trait, and has emerged as a biomarker of aging, health and disease. TL in leukocytes shortens in a divinable way with age by approximately 20–40 base pairs (bp) per year.[4] The aging and subsequent death of cells often happens if the mean TL reaches a critical value.[5]

We proposed a hypothesis that propofol dose can be predicted by TL before operation, which greatly reduced the risk of anesthesia, especially the elderly. Hence, we designed a study to evaluate the association between TL in the DNA of peripheral blood leukocytes (PBLs) and propofol dose in the induction of general anesthesia.

Methods

Ethical approval of the study protocol

This study was approved by Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command and written informed consent was obtained from all subjects participating in the trial. The trial was registered prior to patient enrollment at clinicaltrials.gov (NCT03429309, Principal investigator: WeiFeng Tu, Date of registration: February 9, 2018)

Exclusion criteria

We excluded people: with known cardiac, hepatic, pulmonary, or renal disease; hearing disorders, neurologic diseases, or diabetes mellitus; consuming > 20 g of alcohol daily; with a body mass index (18 $kg.m^{-2}$ > BMI > 30 $kg.m^{-2}$).

Research cohort and indicators

The number of observations should be more than 20 times the number of independent variables in multivariable linear regression analyses. We used four independent variables (TL; Body weight; sex and difference of BIS before and after anesthesia induction) in the study. Therefore, we recruited 100 patients of Chinese Han population aged from 18 to 80 years, with American Society of Anesthesiologists (ASA) physical status I–II. Testing took place in the morning after an overnight fast. Patients were scheduled for elective surgery.

Anesthesia induction

After arrival in the operating theatre, a peripheral venous catheter was inserted for infusion of fluids and drugs. The heart rate, peripheral oxygen saturation, noninvasive blood pressure and Bispectral Index (BIS) were monitored continuously. Also, 100% oxygen was given for 3 minutes by face mask.

The induction of anesthesia was started by infusion of propofol (Fresenius Kabi, Bad Homburg vor der Höhe, Germany) using an intravenous syringe pump (B. Braun Melsungen, Germany) at 30 $mg.kg^{-1}.h^{-1}$. Recording began before propofol infusion was started.

When patients had a BIS of 70, they were asked loudly to ''open their eyes''. This command was repeated up to three times, and the eyelash reflex was examined at 15-s intervals until they became unconscious (i.e., lost response to a verbal command, no spontaneous movements and loss of the eyelash reflex) by the anesthetist. Recording of the heart rate, noninvasive blood pressure, and BIS was done when patients were awake (baseline) until propofol induced unconsciousness. The propofol dose and time taken for consciousness to disappear were recorded after propofol administration. Patients were instructed not to open their eyes, talk, or move during recording of the heart rate, noninvasive blood pressure, and BIS before propofol infusion. The sedation level was assessed until the patient became unconscious.

TL measurement

The quantitative Real Time polymerase chain reaction (qRT-PCR) technique is the method used most frequently by investigators for measuring TL. Blood samples were obtained from 100 patients during surgery. TL of DNA in PBLs pretreatment was assessed by qRT-PCR, as literature described previously.[6,7] Total PBLs were separated using Red Blood Cell Lysis Buffer (C3702; Beyotime Institute of Biotechnology, Beijing, China). Genomic DNA was extracted from PBLs using a DNA Blood kit (Shanghai Majorbio Pharmaceutical Technology, Shanghai, China). qRT-PCR was done in a 7500 Real Time

Table 1 Demographic data.

Research indicators	Characteristics
Patients	n = 100
Age (years)	18–80 (49.66 ± 15.99)
Difference of BIS before and after anesthesia induction	(33.43 ± 18.83)
Body weight (kg)	40-87 (63.13 ± 10.52)
BMI ($kg.m^{-2}$)	30 > BMI > 18 (23.31 ± 3.02)
Sex (female, male)	female = 44, male = 46

PCR system (Applied Biosystems, Foster City, CA). The relative ratio of telomere-repeat copy number to single-copy number (T/S ratio) was calculated. Samples were compared with a reference DNA sample.

Detection of mutation at the 5'end or 3'end of TERC

TERC provides instructions for making one component of the enzyme telomerase.

qRT-PCR was used to detect gene mutation at the 5'end or 3'end of *TERC*, as literature described previously.[8] The reference gene *β-globin* was used for calculation of the copy number of *TERC*. An amplicon of size 1190 bp extending from 433 bp at the 5'end flanking to 306-bp downstream of *TERC* was amplified for *TERC* sequencing. Genomic DNA was obtained with a SYBR™ Premix Ex Taq kit (AK6006; TaKaRa Biotechnology, Shiga, Japan). The individual copy number of *TERC* at the 5'end or 3'end was calculated as the ratio of *TERC*/*β-globin* of each sample using the comparative CT method ($2^{-\Delta\Delta CT}$).[8]

Statistical analyses

Multivariable linear regression analyses were undertaken to assess the relationship between the: propofol dose in anesthesia induction, and TL; sex; Body weight; difference of BIS before and after anesthesia induction, $p < 0.05$ was considered significant. The propofol dose and TL correlation were measured with Pearson. Descriptive data were analyzed by mean ± standard deviation. Analyses were done using SPSS v21.0 (IBM, Armonk, NY, USA).

Results

Demographic data are presented in Table 1.

Association between the propofol dose and other factors

Telomere length in PBLsis 1.11 ± 0.41. The propofol dose in anesthesia induction (118.84 ± 27.58 mg) was clearly correlated significantly with TL (r = 0.78, $p < 0.001$); body weight (r = 0.84, $p = 0.004$); sex (r = 0.83, $p = 0.004$); and difference of BIS before and after anesthesia induction (r = 0.85, $p = 0.029$). By comparing the absolute values of standardized regression coefficients (0.58, 0.21, 0.19, and 0.12) of the four variables (Table 2), it can be seen that TL contributes the most to the propofol dose in anesthesia induction.

Table 2 Statistical results of the relationship between five variables (Coefficients[a]).

Model	Standardized coefficients Beta	t	Sig.
(Constant)		2.450	0.016
Tel	0.578	8.977	0.000
Body weight	0.206	2.984	0.004
Sex	0.189	2.953	0.004
BIS	0.124	2.215	0.029

[a] Dependent Variable: Propofol Dose.

Linear correlation scatter/dot between the propofol dose and other factors

The propofol dose in anesthesia induction was positively correlated with TL; body weight; difference of BIS before and after anesthesia induction from the scatter diagram (Fig. 1).

Mutation at the 5'end or 3'end of TERC

Mutation at the 5'end or 3'end of *TERC* was not found in 100 participants.

Discussion

We found a positive association between TL in DNA in PBLs and the propofol dose in anesthesia induction. Also, the propofol dose decreased with shorter TL (Fig. 1). Our study could reveal a closely association between the propofol dose and age-related outcomes by TL. This strategy could allow us to apply TL to assess changes in tolerance of the body to propofol as people get older. However, the mutation at the 5'end or 3'end of *TERC* (a ribonucleoprotein that contains the RNA template in telomerase) was not found in 100 patients, which showed that telomere in these patients was relatively stable. Hence, we inferred that tolerance of the body to propofol showed strong associations with the inherent genetic factors TL of aging. TL may be a promising predictor of the propofol dose, which is beneficial to individualization of the propofol dose and reduction in the risk of anesthesia.

In addition to TL, although we observed that the propofol dose in anesthesia induction was strongly correlated with body weight, sex, and difference of BIS before and after anesthesia induction in 100 participants (Fig. 1), it can be seen that TL contributes the most to the propofol dose by comparing the absolute values of standardized regression coefficients (0.58, 0.21, 0.19, and 0.12) of the four variables (Table 2). In other words, TL may have the greatest influence on the propofol dose in several factors. It may be possible to

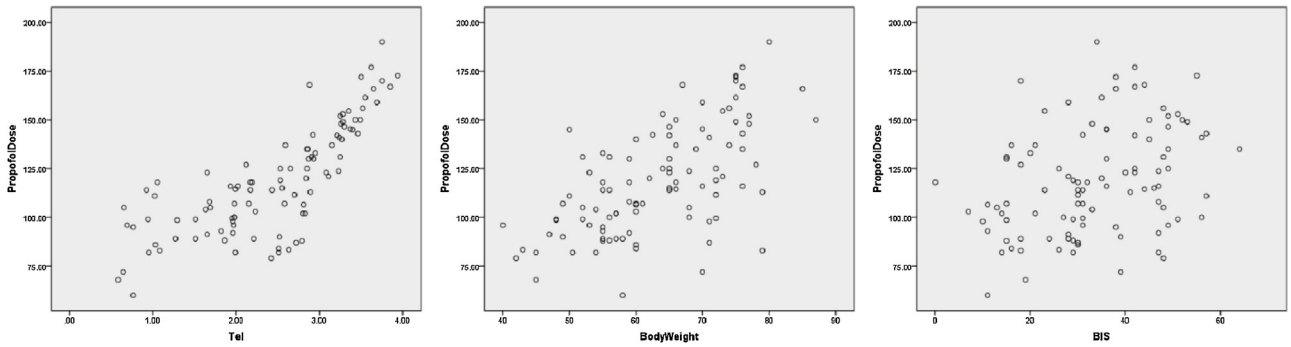

Figure 1 Linear correlation scatter/dot.

use TL association with external disease factors of aging to determine the propofol dose in the future, which can greatly reduce perioperative complications.

This was the first study showing a direct association between the tolerance of the body to the propofol dose and TL as a biomarker. TL has been recognized as a strong and informative biomarker of aging. Several studies have shown that TL in PBLs has an inverse association with age and a robust association with mortality risk score.[9] In particular, Dean et al.[10] observed a strong relationship between shorter TL and increased overall mortality. TL can be used to predict aging-related health outcomes.[9,11] In our study, TL demonstrated associations with propofol dose, which may be helpful for determining the propofol dose during aging as well as improving the safety of anesthesia.

Telomere length and structure may be modulated by genetics, epigenetics, environment and behavioral attitudes.[12] *TERC* was reported to be ubiquitously expressed in different types of normal tissues, and play a vital role in regulating TL. Over-expression of *TERC* increased TL. Mutations in *TERC* are associated with human diseases. Gradual attrition of telomeres occurs during each cell division. The cells become senescent (at least in part), cell-cycle arrest and apoptosis, and cannot divide further when telomeres become very short.[13] Hence, telomeres play a major part in cellular senescence and might contribute significantly to the inherited background of human aging and longevity.[14] Telomere shortening in one tissue may cause systemic effects.[15] Age-matched elderly people with short telomeres in DNA in PBLs have been shown to have worse survival.[16] TL is a predictor of the extent of biological aging and lifespan, or specific for certain biological systems throughout the lifespan. Telomeres shorten with age. The propofol dose decreases with age.[17] The three conform to the dialectical relationship. Aging results from a dynamic, complex, and multifactorial processes related to a decreased propofol dose by gradual accumulation of different types of cellular and molecular damage.[18,19] Given this information, we can infer that TL of inherent heredity factors can reflect the sensitivity of the body to propofol, and may interact with many other relevant factors.

TL dynamics change constantly over a lifespan, but the rate of telomere change may depend upon genetics, environmental and lifestyle-related factors, stochastic factors, and the genetic mutations of telomerase. Previous research has found a significant association between longer TL and better self-rated general health.[20] TL may be an informative biomarker of healthy aging and overall immune competence. Short TL in leukocytes is a cause of impaired immune competence, and has been associated with a higher risk of hospitalization due to infectious disease and infection-related death.[21] Senescence heterogeneity induced by telomere shortening, depends on the initial variance in TL.[22] Hence, TL may be a promising predictor of the propofol dose combined with the physiological status of the body.

However, markers of biological aging may change over a lifespan, and a single biomarker may not be sufficient to reflect aging across various biological systems. It is also clear that many mysteries around telomeres and their function remain. In my study, a key question is to what extent the association between TL and the propofol dose observed in our study was causal. We did not know whether this phenomenon exists among other ethnicity. We did not consider the additional factors, which may have confounded our analyses. Clearly, further studies are needed to evaluate the extent to which TL influences the propofol dose with increasing age. How *TERC* affect telomere length is also our future research direction.

Conclusions

We found a positive association between TL in DNA in PBLs and the propofol dose in anesthesia induction. Also, the propofol dose decreased with shorter TL. This strategy could allow us to apply TL to assess changes in tolerance of the body to propofol as people get older. Hence, TL may be a promising predictor of the propofol dose, which is beneficial to individualization of the propofol dose and reduction in the risk of anesthesia.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Mézière A, Paillaud E, Plaud B. Anesthesia in the elderly. La Presse Médicale. 2013;42:197–201.
2. Spanjer MRK, Bakker NA, Absalom AR. Pharmacology in the elderly and newer anaesthesia drugs. Best Pract Res Clin Anaesthesiol. 2011;25:355–65.
3. Srinivas N, Rachakonda S, Kumar R. Telomeres and Telomere Length: A General Overview. Cancers. 2020;12:1–30.
4. Young AG. The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. Philos Trans R Soc Lond B Biol Sci. 2018;373:1–12.
5. Doksani Y. The Response to DNA Damage at Telomeric Repeats and Its Consequences for Telomere Function. Genes. 2019;10:1–17.
6. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002;30:1–6.
7. Vasilishina A, Kropotov A, Spivak I, et al. Relative Human Telomere Length Quantification by Real-Time PCR. Methods Mol Biol. 2019;1896:39–44.
8. Du HY, Pumbo E, Ivanovich J, et al. TERC and TERT gene mutations in patients with bone marrow failure and the significance of telomere length measurements. Blood. 2009;113:309–16.
9. Herrmann M, Pusceddu I, März W, et al. Telomere biology and age-related diseases. Clin Chem Lab Med. 2018;56:1210–22.
10. Bai XJ. Biomarkes of aging. Adv Exp Med Biol. 2018;1086:217–34.
11. Rode L, Nordestgaard BG, Bojesen SE. Peripheral blood leukocyte telomere length and mortality among 64,637 individuals from the general population. J Natl Cancer Inst. 2015;107:1–8.
12. Rocca MS, Foresta C, Ferlin A. Telomere length: lights and shadows on their role in human reproduction. Biol Reprod. 2019;100:305–17.
13. Shay JW. Telomeres and aging. Curr Opin Cell Biol. 2018;52:1–7.
14. Adwan Shekhidem H, Sharvit L, Leman E, et al. Telomeres and Longevity: A Cause or an Effect? Int J Mol Sci. 2019;20:1–12.
15. Saretzki G. Telomeres, Telomerase and Ageing. Subcell Biochem. 2018;90:221–308.
16. Yuan X, Kronström M, Hellenius ML, et al. Longitudinal changes in leukocyte telomere length and mortality in elderly Swedish men. Aging. 2018;10:3005–16.
17. Olmos M, Ballester JA, Vidarte MA, et al. The Combined Effect of Age and Premedication on the Propofol Requirements for Induction by Target-Controlled Infusion. Anesth Analg. 2000;90:1157–61.
18. Gao X, Zhang Y, Mons U, et al. Leukocyte telomere length and epigenetic-based mortality risk score: associations with all-cause mortality among older adults. Epigenetics. 2018;13:846–57.
19. Dean SG, Zhang C, Gao J, et al. The association between telomere length and mortality in Bangladesh. Aging. 2017;9:1537–48.
20. Smith L, Luchini C, Demurtas J, et al. Telomere length and health outcomes: An umbrella review of systematic reviews and meta-analyses of observational studies. Ageing Res Rev. 2019;51:1–10.
21. Helby J, Nordestgaard BG, Benfield T, et al. Shorter leukocyte telomere length is associated with higher risk of infections: a prospective study of 75,309 individuals from the general population. Haematologica. 2017;102:1457–65.
22. Eugene S, Bourgeron T, Xu Z. Effects of initial telomere length distribution on senescence onset and heterogeneity. J Theor Biol. 2017;413:58–65.