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ORIGINAL ARTICLE

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Differences in the D/L aspartic acid ratios in dentin among different types of teeth from the same individual and estimated age

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Abstract We have studied the correlation between the level of D-aspartic acid in dentin and the period of dentin formation in different types of teeth from the same individual. Except for the third molar, it is generally agreed that the formation of permanent dentin follows the pattern of growth, occurring earliest in the first molar and last in the second molar. In middle-aged individuals, racemization among the different types of teeth was highest in the first molar, corresponding to the earliest period of dentin formation. In elderly individuals, however, racemization tended to be highest in the second molar, in which dentin is formed last during growth. It is assumed that this may be due to the higher ambient temperature of the second molars, which are located deeper in the oral cavity. Thus, these results suggest that in elderly individuals racemization in teeth that have been situated deep in the oral cavity for a long time is more influenced by the environment than by the period of tooth formation.

Keywords Age estimation · Dentin · Aspartic acid racemization · Intraindividual differences

Introduction

D-amino acid is transformed by racemization from L-amino acid in the course of aging. It is accumulated with aging in the tissues in which metabolism proceeds slowly, such as the teeth [1, 2, 3, 4, 5, 6, 7], bone [8, 9, 10, 11, 12, 13], eye lens [14] and brain [15]. In particular, the level of D-aspartic acid in dentin of human teeth generally increases gradually with age. In forensic medicine, the level of D-aspartic acid in dentin is established as a tool for estimating age from teeth [16, 17, 18]. For age estimation, the level of D-aspartic acid en masse in several of the same type of tooth of known age is measured as a standard for legal advice. This control is indispensable, because it is sometimes difficult to keep the same level of precision when measuring actual samples owing to instability in the efficiency of gas chromatography. It is especially important to unify analytical procedures for reproducible gas chromatography [19]. The same type of tooth is used as the reference standard for legal advice because different types of teeth are formed in different periods of growth and the level of D-aspartic acid differs accordingly. There are no reports, however, that have accurately measured and compared the level of D-aspartic acid in dentin of different types of teeth from the same individual.

With regard to the process of dentin formation, it is expected that the earlier the formation of a type of tooth is completed, the higher the level of D-aspartic acid should be. Racemization is a chemical reaction, however, which could be influenced by environmental conditions. Accordingly, it is still unknown whether the measured level of D-aspartic acid in dentin actually agrees with the temporal order of dentin formation.

In this study, we investigated whether the level of D-aspartic acid in dentin is related to the period of formation with respect to each type of tooth in the same individual. We also examined the deviation of the estimated age from the real chronological age on the basis of the measured extent of aspartic acid racemization in dentin.

Materials and methods

The material consisted of a total of 56 teeth, which were obtained from 9 cadavers donated after death at the ages of 58–88 years. Between five and seven different types of tooth, except for the third

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molar, were obtained from each cadaver. As samples for analysis, we selected the teeth in which more than three-quarters of the dentin was retained. We cut longitudinal sections through the specimens, and those sections containing only dentin were collected with a cutter. The sections were rinsed sequentially for 5 min each in water, ethanol and ether in an ultrasonic bath, then dried and pulverized and 10 mg of the pulverized material was used for measurement. D-aspartic acid was analyzed and isolated by the conventional method of gas chromatography [3, 4], on an FS capillary column (length: 25 m, internal diameter: 0.3 mm; G. L. Science, Tokyo) coated with Chirasil-Val [19]. The extent of racemization was obtained by substituting D and L of the following equation Eq 1 with the areas under the respective D-aspartic acid and L-aspartic acid peaks in the chromatograph:

$$\ln[(1 + D/L)/(1 - D/L)] \quad (1)$$

The extent of racemization was determined by averaging the values obtained in 2–4 measurements. For comparison of the extent of racemization among different types of tooth, the homonymous tooth was classified as the same type of tooth, regardless of its location in the upper or lower dentition or on the right or left side.

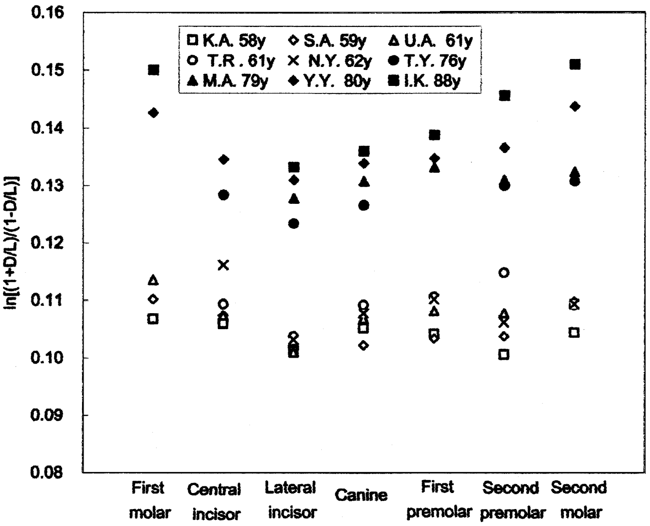

Fig. 1 Differences in the correlation of the D/L ratio with age observed using tooth samples from subjects aged between 58–62 years and 76–88 years. Types of tooth on the x-axis are arranged from left to right according to their order of formation

Results and discussion

Racemization is a chemical reaction in which the L-enantiomer is transformed into the D-enantiomer, or vice versa. This reaction is often detected in the tissues that have a slow metabolism. Although the level of D-amino acid is assumed to increase steadily in a constant environment, the racemization process depends strongly on certain aspects of the environment [20]. For example, it has been reported that racemization is bound up tightly with ambient temperature [21, 22, 23].

We previously investigated the correlation between the level of D-aspartic acid in enamel, dentin and cementum and aging, and found that this correlation was highest between the levels of D-aspartic acid in dentin and aging [24]. It is assumed that this is because dentin is surrounded by enamel and cementum, therefore its water content is kept constant with water supply through the dentinal tubules. We found, however, that racemization proceeds most rapidly in cementum and most slowly in enamel [24]. We assume that this may be due to differences in the ambient temperature.

It is generally agreed that on the whole the permanent dentin is formed in the following temporal order: first molar, central incisor, lateral incisor, canine, first premolar, second premolar and second molar [25]. If we assume that the oral cavity is a uniform environment, D-aspartic acid is expected to be highest in the type of tooth that completes formation in the early period of growth. In K. A. (age at death: 58 years), S. A. (age at death: 59 years) and U. A. (age at death: 61 years), the extent of racemization was highest in the first molar, consistent with the temporal order of formation (Fig. 1). In the elderly group of T. Y. (age at death: 76 years), Y. T (age at death: 80 years) and I. K. (age at death: 88 years), however, the extent of racemization was highest in the second molar, which is formed in the last period of growth, along with the first molar, which is formed in the earliest period of growth (Fig. 1). This may be due to a higher temperature in the molar region deep in the oral cavity than in the front teeth region. The interconversion of aspartic acid enantiomers is a reversible first order chemical reaction. Therefore, the temperature probably highly influences D/L ratios. It is assumed that when a tooth is situated in the oral cavity for longer durations, as in elderly people, the influence of ambient temperature becomes stronger and the influence of the period of tooth formation becomes weaker. Thus, strictly considered, our data suggest that the intraoral environment of the tooth differs between the front teeth region and the molar region (Fig. 1).

In the four subjects from whom all seven types of tooth were obtained, the standard deviation of the extent of racemization of each type of tooth increased in parallel with an increase in age. Thus, these results suggest that the difference in the extent of racemization of each type of tooth increases with age.

The mean level of D-aspartic acid of each type of tooth decreased in the following order: first molar >second molar >second premolar >first premolar >canine >central in-

Table 1 Equations deduced by the method of least squares from respective too the type and all types of tooth

Type of tooth	Y	n	r	k
Central incisor	756.63X–22.20	7	0.980	0.000634
Lateral incisor	780.70X–19.70	9	0.986	0.000622
Canine	806.23X–25.49	9	0.985	0.000601
First premolar	782.58X–23.79	8	0.989	0.000625
Second premolar	677.96X–11.75	9	0.982	0.000711
First molar	686.57X–16.37	5	0.996	0.000723
Second molar	640.76X–9.01	9	0.986	0.000759
Total teeth	696.20X–13.84	56	0.961	0.000665

Y age; X, ln[(1+D/L)/(1–D/L)]
r correlation coefficient
k rate constant of racemization

Table 2 Difference between chronological and estimated ages from each individual types (n=5–9) of tooth

Type of tooth	Difference						
	±0 years	±1 years	±2 years	±3 years	±4 years	±5 years	Over ±6 years
Central incisor	2	2	2	1			
Lateral incisor	3	2	3		1		
Canine	2	4	1	1	1		
First premolar	1	5	1		1		
Second premolar	3	1	4			1	
First molar	2	1	2				
Second molar	2	3	3		1		
Total (%)	15 (26.8)	18 (32.1)	16 (28.6)	2 (3.6)	4 (7.1)	1 (1.8)	0

cisor>lateral incisor which was not necessarily consistent with the temporal order of formation of different types of tooth. This result indicates that the intraoral environment surrounding a particular type of tooth can exert a stronger influence on racemization than can the period of formation (Fig. 1).

In forensic medicine, dentin is used for estimating age from the teeth [16, 17, 18]. Accordingly, by the least square method we calculated the equation for the linear regression line defining the relationship between the extent of racemization and age, with respect to each type of tooth (n=5–7, r=0.980–0.996) and all types of teeth (n=56, r=0.961). We calculated the estimated age by substituting the D/L ratio in the equations deduced by the method of least squares with the measured D/L values of the respective tooth type and all types of tooth (Table 1).

Among the different types of teeth, k was largest in the second molar. For the other types of teeth, k became smaller in the following order: first molar >second premolar >central incisor >first premolar >lateral incisor >canine. Thus, these results reflect the tendency that the deeper in the oral cavity a type of tooth is located, the higher the rate of racemization. It is assumed that the ambient temperature is higher in deeper regions in the oral cavity. It is unlikely that those differences in D/L ratios were caused by variations in the protein composition because we carefully dissected out only dentin and obtained 10 mg pulverized samples where the protein composition was probably homogeneous. However, we still exclude such a possibility.

We calculated the estimated age by the equations (Table 1) for each respective type of tooth, and found that the estimated age was equal to the real chronological age in 15 out of 56 cases. The error range of ±1 year-level contained 18 cases, ±2 years level contained 16 cases, ±3 years level contained 2 cases and more than ±4 years level contained 5 cases out of 56 (Table 2). Likewise, the age here was estimated by the equation for all types of tooth. The estimated age here was equal to the chronological age in 9 out of 56 cases. The error range of ±1 year level contained 19 cases, ±2 years level contained 10 cases, ±3 years level contained 6 cases, and more than ±4 years level contained 12 cases out of 56 (Table 2). Thus, these results confirm that age estimation based the extent of racemization in a specific type of tooth yields a better result than estimations from different types of tooth.

References

1. Helfman PM, Bada JL (1975) Aspartic acid racemisation in tooth enamel from living humans. Proc Natl Acad Sci USA 72:2891–2894
2. Helfman PM, Bada JL (1976) Aspartic acid racemisation in dentine as a measure of ageing. Nature 262:279–281
3. Ogino T, Ogino H (1988) Application to forensic odontology of aspartic acid racemization in unerupted and supernumerary teeth. J Dent Res 67:1319–1322
4. Ohtani S, Yamamoto K (1991) Age estimation using the racemization of amino acid in human dentin. J Forensic Sci 36: 792–800
5. Ritz S, Schütz HW, Peper C (1993) Postmortem estimation of age at death based on aspartic acid racemization in dentin: its applicability for root dentin. Int J Legal Med 105: 289–293
6. Carolan VA, Gardner MLG, Lucy D, Pollard AM (1997) Some considerations regarding the use of amino acid racemization in human dentine as an indicator of age at death. J Forensic Sci 42:10–16
7. Waite ER, Collins MJ, Ritz ST, Schütz HW, Cattaneo C, Borrman HIM (1999) A review of the methodological aspects of aspartic acid racemization analysis for use in forensic science. Forensic Sci Int 103:113–124
8. Ritz S, Turzynski A, Schütz HW (1994) Estimation of age at death based on aspartic acid racemization in noncollagenous bone proteins. Forensic Sci Int 69:149–159
9. Ritz S, Turzynski A, Schütz HW, Hollmann A, Rochholz G (1996) Identification of osteocalcin as a permanent aging constituent of the bone matrix: basis for an accurate age at death determination. Forensic Sci Int 770:13–26
10. Pfeiffer H, Mörnstad H, Teivens A (1995) Estimation of chronologic age using the aspartic acid racemization method. 1. On human rib cartilage. Int J Legal Med 108:19–23
11. Pfeiffer H, Mörnstad H, Teivens A (1995) Estimation of chronologic age using the aspartic acid racemization method. 2. On human rib cortical bone. Int J Legal Med 108:24–26
12. Ohtani S, Matsushima Y, Kobayashi Y, Kishi K (1998) Evaluation of aspartic acid racemization ratios in the human femur for age estimation. J Forensic Sci 43:949–953
13. Ohtani S, Matsushima Y, Kobayashi Y, Yamamoto T (2002) Age estimation by measuring the racemization of aspartic acid from total amino acid content of several types of bone and rib cartilage: a preliminary account. J Forensic Sci 47:32–36
14. Fujii N, Ishibashi Y, Satoh K, Fujino M, Harada K (1994) Simultaneous racemization and isomerization at specific aspartic acid residues in αB-crystallin from the aged human lens. Biochem Biophys Acta 1204:157–163
15. Man EH, Sandhouse ME, Burg J, Fisher GH (1983) Accumulation of D-aspartic acid with age in the human brain. Science 220:1407–1408
16. Ohtani S (1995) Estimation of age from the teeth of unidentified corpses using the amino acid racemization method with reference to actual cases. Am J Forensic Med Pathol 16:238–242

17. Ritz ST, Cattaneo C, Collins MJ, Waite ER, Schütz HW, Kaatsch HJ, Borrman HIM (2000) Age estimation: the state of art in relation to the specific demands of forensic practise. Int J Legal Med 113:129–136
18. Ritz ST, Rochholz G, Schütz HW, Collins MJ, Waite ER, Cattaneo C, Kaatsch HJ (2000) Quality assurance in age estimation based on aspartic acid racemisation. Int J Legal Med 114: 83–86
19. Abe I, Kuramoto S, Musha S (1983) Chiral phases derived from XE-60 for glass capillary gas chromatography of amino acid enantiomers. J Chromatogr 258:35–42
20. Bada JL, Kvenvolden KA, Peterson E (1973) Racemization of amino acids in bones. Nature 73:245:308–310
21. Schroeder RA, Bada JL (1973) Glacial-postglacial temperature difference deduced from aspartic acid racemization in fossil bones. Science 182:479–482
22. Bada JL, Schroeder RA (1975) Amino acid racemization reactions and their geochemical implications. Naturwissenschaften 62:71–79
23. Ohtani S (2002) Technical notes for age estimation using the femur: influence of various analytical conditions on D-aspartic acid contents. Int J Legal Med 116:361–364
24. Ohtani S (1995) Studies on age estimation using racemization of aspartic acid in cementum. J Forensic Sci 40:805–807
25. Logan WHG, Kronfeld R (1933) Development of the human jaws and surrounding structures from birth to the age of fifteen years. J Am Dent Assoc 20:379–427