PI SUBTYPING IN DENTAL PULPS

Akira KIDO and Masakazu OYA

Department of Legal Medicine, Yamanashi Medical University, Yamanashi-ken 409-38, Japan

INTRODUCTION

Genetic polymorphism of human serum α_1-antitrypsin (PI) was first discovered by Fagerhol and Braend (1965) using acid starch gel electrophoresis. The common PI M type can be separated into six subtypes with three alleles, PI*M1, PI*M2 and PI*M3, by means of isoelectric focusing followed by direct protein staining with Coomassie Brilliant Blue (CBB) (Frants and Eriksson, 1976).

Recent introduction of the technique of immunoblotting has provided much better visualization of focused PI than the CBB staining method (Whitehouse et al., 1989). In the present study, PI subtyping was attempted from dental pulps using isoelectric focusing followed by immunoblotting.

MATERIALS AND METHODS

Teeth were collected from 43 patients who received treatment at the Dental Clinic of Yamanashi Medical University Hospital. Fifteen samples were examined immediately after extraction and the remaining 28 samples after storage at room temperature for various periods of week. Serum samples were also taken from the same 43 subjects as control. The tooth was crushed with a hammer, and the dental pulp was picked out from the pulp cavity. The pulp tissue weighing 10 to 20 mg was macerated in 30 μl distilled water and mashed with a glass rod. Eight μl of dental pulp lysates or serum samples were treated with 1 μl 0.1 M dithiothreitol for 30 min at room temperature and then with 1 μl 0.1 M iodoacetamide for 60 min at 4°C. Serum samples were diluted 1:20, but dental pulp samples were not diluted.

The amount of PI present in the dental pulp was quantitated using rocket immunoelectrophoresis (Laurell, 1966).

Isoelectric focusing was performed using a Bio-Phoresis Electrophoresis Cell (Bio-Rad) and a Model 3000 Xi Power Supply (Bio-Rad). Polyacrylamide gels (230 x 110 x 0.5 mm) were prepared with 20 ml acrylamide stock solution (5.25% acrylamide/0.25% N,N'-methylenebisacrylamide) containing 1 ml Pharmalyte pH 4.2-4.9 (Pharmacia), 0.3 ml 0.01% riboflavin, 2.5 g sucrose and 0.3 g N-(2-acetamide)-2-aminoethanesulfonic acid. The electrode paper strips were soaked with 1 M phosphoric acid for the anode and with 1 M sodium hydroxide for the cathode. After prefocusing at 1500 Vmax and 5 mAmax for 60 min, 10 μl of the samples was applied to the gel surface 2 cm from the cathode using 5 x 6 mm filter paper strips (Whatman No. 3). Electrofocusing was con-

ducted at a constant voltage of 1500 V for 180 min. The sample applicators were removed after 60 min of focusing. During focusing the gel was cooled by circulating water at 4°C.

A sheet of nitrocellulose membrane (Bio-Rad) was placed on the gel surface and left for 60 min with 1 kg weight. Following blotting the membrane was rinsed in 20 mM Tris/500 mM sodium chloride buffer, pH 7.5, (TBS) for 10 min and immersed in TBS containing 3% gelatin for 30 min. After washing in TBS for 15 min, the membrane was incubated for 60 min in rabbit anti-human PI serum (DAKO) that was diluted 500-fold with TBS containing 0.05% Tween 20 (TTBS). Then the membrane was washed 3 times in TTBS for 30 min and incubated for 60 min in goat anti-rabbit IgG serum conjugated with alkaline phosphatase (Sigma) that was diluted 500-fold with TTBS. After 3 washes in TTBS for 30 min, the membrane was incubated in 50 ml of a staining solution (1.8 g sodium hydroxide and 3.7 g boric acid/1000 ml) containing 25 mg β-naphthyl phosphate, 25 mg Fast Blue BB salt and 60 mg magnesium sulfate for a few minutes.

RESULTS AND DISCUSSION

The amount of PI present in dental pulp lysates from 15 fresh tooth samples was determined by rocket immunoelectrophoresis. The values ranged from 0.29 to 1.90 mg/ml. The mean value was 0.82±0.11 mg/ml, which was about one third of the value in normal serum (2.42 mg/ml) (Blundell and Frazer, 1975).

Table 1 summarizes the results of PI subtyping in fresh teeth and in teeth stored at room temperature for various periods.

The PI patterns in fresh dental pulps were identified as clearly and intensely as those in serum samples, and the types observed in dental pulps agreed with those in the corresponding serum samples (Fig. 1). All the dental pulp samples examined were correctly subtyped after storage for up to 4 weeks. The pattern in dental pulps from teeth stored for 1 week at room temperature is shown in Fig. 2.

Table 1. Positive results of PI subtyping in dental pulps

Periods of storage	No. tested	Subtype			
		M1	M1M2	M2	M2M3
Fresh	15	9	5	1	
1 week	10	5	3	1	1
2 weeks	6	4	1		1
3 weeks	6	5	1		
4 weeks	6	4	2		

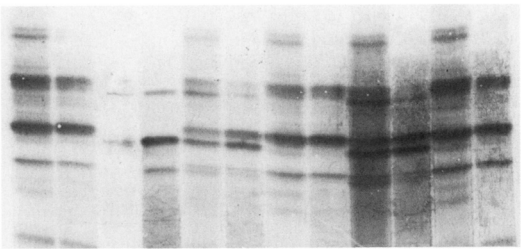

Fig. 1. Isoelectric focusing PI pattern in fresh dental pulps (DP) and the corresponding serum samples (S). 1: M1, 2: M2, 3: M1M2, 4: M1, 5: M1M2, 6: M1. The anode is at the top.

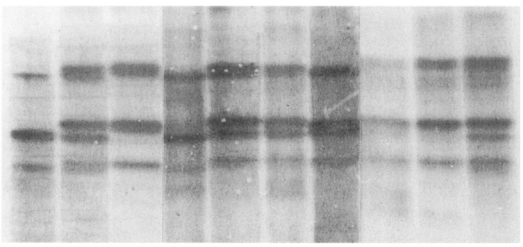

Fig. 2. Isoelectric focusing PI pattern in control sera (1–3) and dental pulps from teeth stored for 1 week at room temperature (4–10). 1: M2, 2: M1M2, 3: M1, 4: M2, 5: M1M2, 6: M1M2, 7: M2M3, 8: M1, 9: M1, 10: M1M2. The anode is at the top.

In conclusion, isoelectric focusing followed by immunoblotting permits reliable PI subtyping from teeth stored for up to 4 weeks and is therefore recommended to be used in medico-legal practice. The PI subtyping combined with the TF and GC typings is useful for personal identification of teeth, particularly in cases of mass disasters such as automobile accidents, aircraft crashes and explosions.

REFERENCES

Blundell G. and Frazer A. (1975) Alpha$_1$ antitrypsin phenotypes in Northern Ireland. *Ann. Hum. Genet.*, 38, 289–294.

Fagerhol M.K. and Braend M. (1965) Serum prealbumin: Polymorphism in man. *Science*, 149, 986–987.

Frants R.R. and Eriksson A.W. (1976) α_1-Antitrypsin: Common subtypes of Pi M. *Hum. Hered.*, 26, 435–440.

Laurell C.B. (1966) Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.*, 15, 45–52.

Whitehouse D.B., Lovegrove J.U. and Hopkinson D.A. (1989) Variation in alpha-1-antitrypsin phenotypes associated with penicillamine therapy. *Clin. Chim. Acta*, 179, 109–116.