Transferrin Subtyping in Human Organ Tissues

Akira KIDO, Yukiko KIMURA and Masakazu OYA

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

INTRODUCTION

Serum transferrin (TF) exhibits genetic polymorphism with 2 codominant alleles, TF*C1 and TF*C2 (Kühnl and Spielmann 1978, Thymann 1978). By means of sensitive immunoblotting techniques, the TF subtypes have been demonstrated also in bloodstains (Yamaba *et al.* 1991), semen (Yamaba *et al.* 1991), urine (Kishi *et al.* 1990) and dental pulps (Kido *et al.* 1993). In this study we report the results of TF subtyping in human organ tissues using immunoblotting after isoelectric focusing.

MATERIALS AND METHODS

Following tissue materials were obtained from 13 cadavers (11 males, 2 females) who were medicolegally autopsied within 48 h after death: spleen, pancreas, heart (cardiac muscle), liver, muscle (m. rectus abdominis), lung, kidney, skin including adipose tissue, brain, prostate, testis, ovary and uterus. 5 g of the tissue sample was stored in a glass bottle at room temperature and examined at 1-week intervals. The tissue weighing 0.5 g was homogenized in 0.5 ml of distilled water using an Ultra-Turrax homogenizer (Janke & Kunkel KG, Staufen in Breisgau, FRG). The homogenate was centrifuged at 10,000 rpm for 60 min and the supernatant was retained for analysis. Blood samples were also taken from the same subjects. Quantitation of TF was carried out by the method of rocket immunoelectrophoresis reported previously (Kido *et al.* 1993).

8 µl of the supernatant or serum was treated with 2 µl of 1 M potassium phosphate buffer (pH 7.0) containing 50 U/ml neuraminidase from *Clostridium perfringens* (type V, Sigma, USA) for 18 h at room temperature. The desialated supernatant was diluted 1:20 and the desialated serum 1:100 with distilled water. They were applied to the gel using 5 x 6 mm filter paper strips (Whatman No. 3, UK). Isoelectric focusing and immunoblotting were described in our previous report (Kido *et al.* 1993).

RESULTS AND DISCUSSION

The amount of TF in supernatants of various human organ

tissue homogenates was estimated by rocket immunoelectrophoresis. The mean value (μg/ml) was: spleen 342±87, pancreas 143±20, heart 251±11, liver 132±40, muscle 111±8, lung 568±113, kidney 259±81, skin 162±28, brain 69±4, prostate 311±22, testis 346±68, ovary 631±167 and uterus 601±40. These values are equivalent to 1/40 - 1/4 of the amount of TF in human serum. A typical example of rocket immunoelectrophoresis of cardiac tissues is shown in Fig. 1.

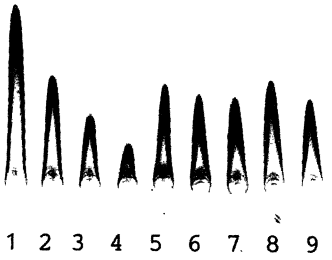

Fig. 1. Rocket immunoelectrophoresis of cardiac tissues in 5 cases. 1-4: serially diluted TF references (1: 134 μg/ml, 2: 67 μg/ml, 3: 33 μg/ml, 4: 17 μg/ml), 5-9: 5-fold diluted cardiac supernatants (5: 60 μg/ml, 6: 53 μg/ml, 7: 51 μg/ml, 8: 63 μg/ml, 9: 49 μg/ml). The anode is at the top.

Table 1 summarizes the results for the determination of TF subtypes in fresh and stored tissues. In fresh tissues of

Table 1. Positive results for the determination of TF subtypes from various human organ tissues

Phenotype	No. tested	Period of storage	Tissue												
			Spleen	Pancreas	Heart	Liver	Muscle	Lung	Kidney	Skin	Brain	Prostate	Testis	Ovary	Uterus
C1	8	48 hours	8	4	8	8	8	8	8	6	8	7	7	1	1
	(M: 7, F: 1)	1 week	6	3	8	5	8	8	8	4	8	7	7	1	1
		2 weeks	2	0	6	3	5	6	5	1	2	3	1	1	1
		3 weeks	2		3	0	3	4	3	1	2	3	1	1	1
		4 weeks	0		2		1	3	3	1	0	0	1	0	1
C2-1	4	48 hours	4	2	4	4	4	4	4	3	4	3	3	1	1
	(M: 3, F: 1)	1 week	2	0	4	2	4	4	4	0	4	3	3	1	1
		2 weeks	2		1	0	2	2	3		2	2	2	0	0
		3 weeks	2		1		2	2	2		2	1	2		
		4 weeks	0		0		1	1	2		1	1	1		
C2	1	48 hours	1	0	1	1	1	1	1	1	1	1	1		
	(M: 1)	1 week	1		1	1	1	1	1	0	1	1	1		
		2 weeks	1		1	1	1	1	1		1	1	1		
		3 weeks	0		1	0	1	1	1		0	1	1		
		4 weeks			1		0	1	1			0	1		

M: male, F: female

almost all the organs examined clear TF subtype patterns were obtained except 7 samples of the pancreas and 3 samples of the skin. The pattern in organ tissues agreed completely with that in the corresponding serum samples (Fig. 2). When stored for 1 week, 3 common TF subtypes were correctly determined from tissues of heart, muscle, lung, kidney, brain, prostate, testis, ovary and uterus. The bands became fainter and more indistinct with increasing period of

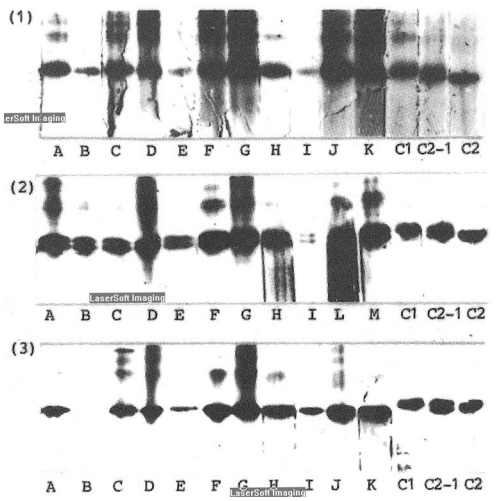

Fig. 2. Isoelectric focusing patterns of TF subtypes in fresh human tissues. (1) male, TF C1; (2) female, TF C2-1; (3) male, TF C2. A: spleen; B: pancreas; C: heart; D: liver; E: muscle; F: lung; G: kidney; H: skin; I: brain; J: prostate; K: testis; L: ovary; M: uterus; C1: serum sample for TF C1; C2-1: serum sample for TF C2-1; C2: serum sample for TF C2. The anode is at the top.

storage. No significant difference in loss of intensity was observed among the allelic products. Storage for 2 weeks yielded much poorer results and it was difficult to identify the TF bands in most organ tissues.

In mass disaster such as automobile accidents, aircraft crashes or explosions, human organs are often submitted for personal identification. The TF subtype system can be a useful means for medicolegal identification of human organs in such cases.

REFERENCES

Kido A, Kimura Y, Oya M (1993) Transferrin subtyping in dental pulps. J Forensic Sci (in press)

Kishi K, Ikehara Y, Yasuda T, Mizuta K, Sato W (1990) Transferrin polymorphism detected in human urine using isoelectric focusing followed by immunoblotting. Forensic Sci Int 45: 225-230

Kühnl P, Spielmann W (1978) Transferrin: Evidence for two common subtypes of the Tf^C allele. Hum Genet 43: 91-95

Thymann M (1978) Identification of a new serum protein polymorphism as transferrin. Hum Genet 43: 225-229

Yamaba T, Sawazaki K, Yasuda T, Nadano D, Iida R, Kishi K (1991) Transferrin subtyping of bloodstains and semen using isoelectric focusing and immunoblotting. Forensic Sci Int 50: 187-194