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Chapter 8

METABOLISM OF NORMAL AND MET30 TRANSTHYRETIN

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I. INTRODUCTION

Hereditary amyloidosis is a heterogeneous disease characterized by systemic or localized deposition of fibrillar proteins which invade the extracellular spaces of organs, destroying normal tissue architecture and function. These amyloid deposits demonstrate green birefringence under polarization microscopy when stained with Congo red, and this unique characteristic is used in diagnosis. The first amyloid protein identified (Costa *et al.*, 1978) and the one most commonly involved in hereditary amyloidosis is transthyretin (TTR). Normal TTR is a 55-kDa soluble plasma protein (20–40 mg/dl) which consists of four identical subunits held together noncovalently. TTR is synthesized in the liver and serves as a carrier for thyroxin and the retinol:retinol-binding protein complex. Over 40 amino acid mutations of TTR have been found related to hereditary amyloidosis, but the most common is the substitution of methionine for valine at position 30. This single amino acid substitution, found worldwide, gives rise to familial amyloidotic polyneuropathy Type I (FAP I).

FAP is an autosomal dominant disease in which amyloid deposits show a systemic distribution in the peripheral and autonomic nervous, cardiovascular, and renal systems. Although the mutant protein is present from birth, FAP shows a delayed onset of symptoms until the third to seventh decade of life, with death usually occurring 10–15 years following onset of disease. Both individuals with FAP (Benson and Dwulet, 1983; Skinner *et al.*, 1985; Westermark *et al.*, 1985) and at risk nonaffected carriers of the Met30 gene (Shoji and Nakagawa, 1988) have shown lower than normal serum transthyretin levels. One explanation could be increased metabolic turnover of TTR due to the mutation; although no turnover studies have been done with the mutant proteins. The purpose of this study was to elucidate the metabolism of both normal and Met30 variant TTR in a normal and an FAP-affected individual using isotopic tracer techniques and computer-aided kinetic modeling. Knowledge of the kinetics of normal and variant TTR metabolism may help in understanding amyloid fibril formation and give direction for possible prevention.

II. METHODS

Laboratory methods and clinical procedures for collection of data were previously reported from our laboratory (Murrell, 1992). Purified native normal and Met30 variant TTR from a homozygous individual were iodinated with ^{131}I- and ^{125}I-labeled monochloride, respectively, and then purified by size exclusion chromatography. For the clinical experiment two human

TABLE I

PATIENT AND DOSING INFORMATION

Subject	Age	Sex	Weight (kg)	^{131}I dose (μCi)	TTR (μg)	Met30 TTR (μg)	^{125}I dose (μCi)
Normal control	61	Female	69.5	22	15	15	35
FAP affected	68	Male	78.1	22	15	15	35

subjects, a normal control and an affected FAP individual, were simultaneously administered both isotopically labeled proteins intravenously. Blood and 24-hr urine samples were collected for 7 days. Table I shows specific patient dosing data. Plasma and urine aliquots were assayed for radioactivity.

The data obtained from clinical study were analyzed through computer-aided kinetic modeling. The time course of plasma decay and appearance in urine of the radiolabels was plotted for each subject and then functions were derived and a kinetic model was developed to simultaneously fit the

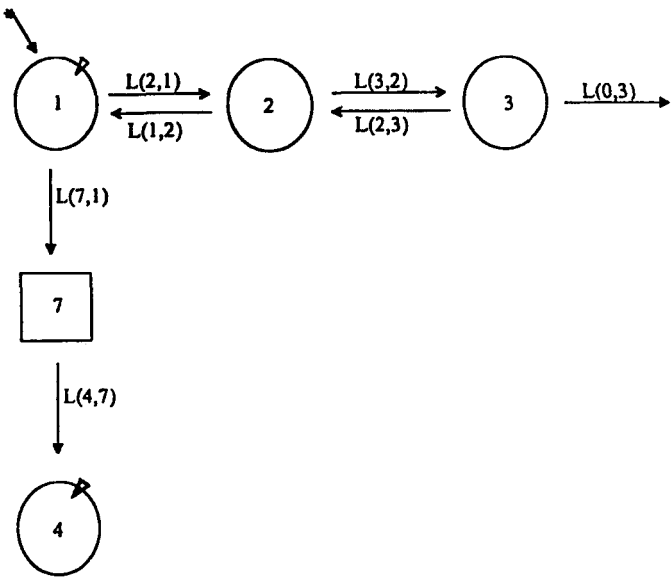

FIG. 1. Kinetic model for ^{131}I and ^{125}I plasma decay and urinary excretion. Compartment 1 represents plasma; compartment 7, a delay compartment, compartment 4, urine; and compartments 2 and 3, undetermined. The asterisk indicates the site of introducing tracer and the small triangles indicate the sites of sampling.

plasma and urine data using the NIH program SAAM, originally introduced by Berman in 1963. TTR plasma residence times were obtained from the areas under the plasma decay curves, and fractional catabolic rate calculated as the reciprocal of the residence time. Plasma volume was initially calculated as 4.5% of total body weight and then allowed to vary to give the best fitting curve.

III. RESULTS

Figure 1 shows the kinetic model and Table II the set of parameters which simultaneously described the plasma decay of ^{131}I- and ^{125}I-Met30-TTR and the appearance of radiolabel in the urine. The radiolabels showed partial exchange with two undetermined body compartments, but a net flux back into the plasma compartment, and then disposal in urine following a 24-hr delay. Figures 2 and 3 show the modeled curves for both the normal control and FAP-affected individual, respectively. Between 80 and 90% of

TABLE II

PARAMETERS USED IN THE MODEL AND METABOLISM VALUES

	Normal subject		FAP subject	
	^{131}I (wild type)	^{125}I (Met30 variant)	^{131}I (wild type)	^{125}I (Met30 variant)
Initial conditions[a] (@ 10 min)				
IC(1)	0.599	0.498	0.652	0.568
IC(4)	0.401	0.502	0.348	0.432
Rate coefficients (hr^{-1})				
$L(2,1)$	4.20E-2	6.23E-2	6.84E-2	1.75E-1
$L(1,2)$	5.21E-2	5.84E-2	8.37E-2	1.85E-1
$L(3,2)$	1.24E-2	1.58E-2	1.98E-2	4.28E-2
$L(2,3)$	4.18E-3	7.80E-3	7.60E-3	1.79E-2
$L(0,3)$	0	0	0	5.99E-3
$L(7,1)$	2.32E-2	2.99E-2	2.50E-2	3.55E-2
$L(4,7)$	1	1	1	1
Delay parameters (hr.)				
DT(7)	23.8	23.8	22.7	22.7
DN(7)	4	4	4	4
Metabolic values				
Residence time (hr)	25.77	16.64	26.04	12.40
Fractional catabolic rate (hr^{-1})	0.039	0.060	0.038	0.081

[a] The model does not account for the initial rapid excretion.

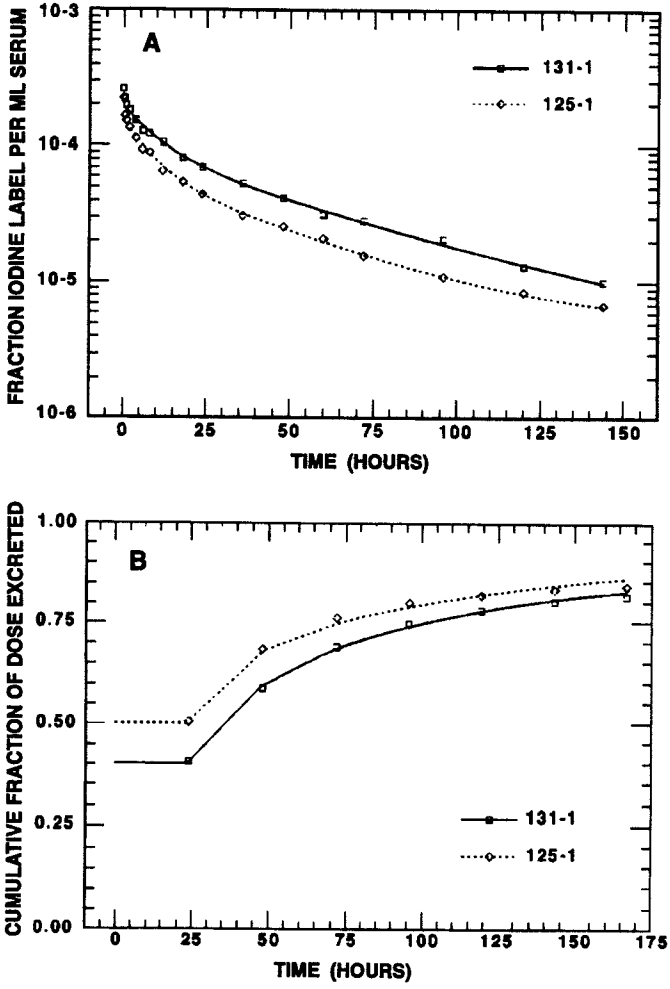

FIG. 2. (A) Plasma ^{131}I and ^{125}I decay, normal subject. (B) Cumulative fraction of dose excreted.

the radiolabels were recovered in urine within 7 days following iv administration of the labeled proteins, with as much as 50% present within the first 10 min for both subjects. TCA precipitation of urine aliquots showed over 95% of the isotope was not associated with protein (Murrell, 1992).

Table I gives specific patient information and Table II the metabolism values derived through modeling the curves in Figs. 2 and 3. Both subjects metabolized and excreted ^{125}I faster than ^{131}I, 1.5-fold faster in the normal

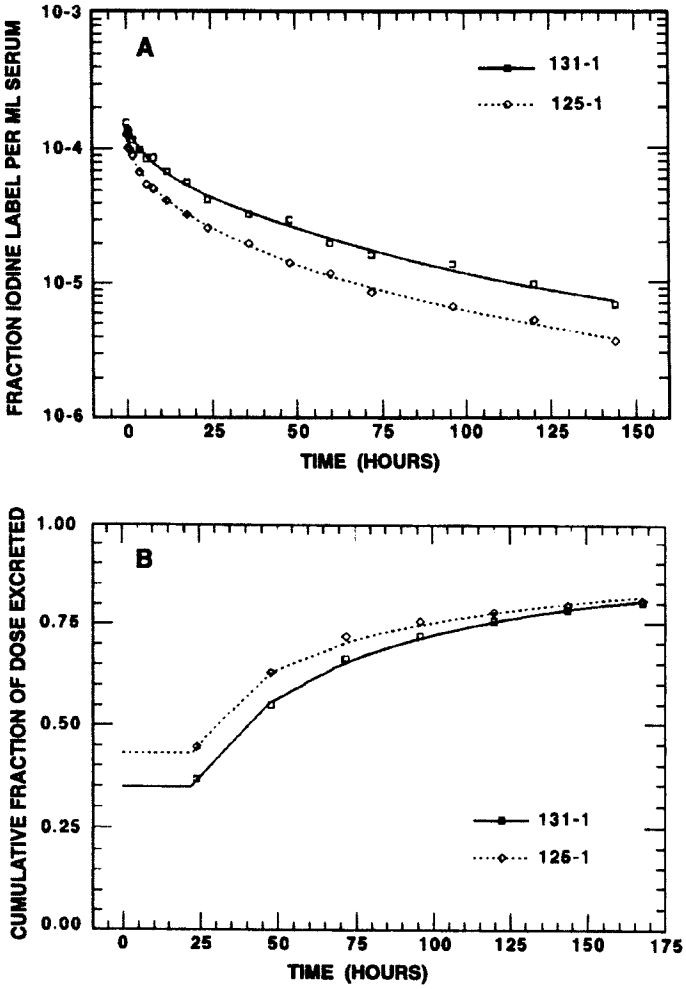

FIG. 3. (A) Plasma ^{131}I and ^{125}I decay, FAP subject. (B) Cumulative fraction of dose excreted.

and 2.1-fold faster in the FAP-affected individual. Residence time of ^{131}I was approximately 26 hr for both subjects, but the residence time of ^{125}I for the FAP-affected subject was 75% (12.4 hr) of that for the normal control (16.6 hr).

IV. DISCUSSION

The results of this study show that the ^{125}I-Met30-TTR is metabolized at a faster rate than normal ^{131}I-TTR regardless of the subject's medical

status. This finding is in agreement with the suggestion by Hamilton *et al.* (1992) that the structural change in the Met30 variant of transthyretin may affect the metabolic properties of the mutant protein. In addition, several investigators have found overall serum TTR levels to be significantly lower in amyloidosis patients compared to normal controls, without distinction between normal and variant protein (Benson and Dwulet, 1983; Westermark *et al.,* 1985; Shoji and Nakagawa, 1988). This study showed the residence time of ^{125}I was lower but that of ^{131}I was equal in the FAP-affected individual compared to the normal control, indicating that only the variant protein is metabolized faster in the disease state. Thus, it is possible that both the structural changes in variant TTR and the medical status of the individual contributed to the increased rate of metabolism of Met30-TTR in the FAP affected individual. These results will contribute to our understanding of amyloid fibril formation *in vivo* for FAP-affected individuals. Future studies will involve more subjects, including unaffected carriers of the Met30-TTR gene.

REFERENCES

Benson, M. D., and Dwulet, F. E. (1983). Prealbumin and retinol binding protein serum concentrations in the Indiana type hereditary amyloidosis. *Arthritis Rheum.* **26,** 1493–1498.

Berman, M. (1963). A postulate to aid in model building. *J. Theor. Biol.* **4,** 229–236.

Costa, P. P., Figueira, A. S., and Bravo, F. R. (1978). Amyloid fibril protein related to prealbumin in familial amyloidotic polyneuropathy. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 4499–4503.

Hamilton, J. A., Steinrauf, L. K., Liepnieks, J., Benson, M. D., Holmgren, G., Sandgren, O., and Steen, L. (1992). Alteration in molecular structure which results in disease: The Met30-variant of human plasma transthyretin. *Biochim. Biophys. Acta* **1139,** 9–16.

Murrell, J. R. (1992). Ph.D. Thesis, Indiana University, School of Medicine, Department of Medical and Molecular Genetics, Indianapolis.

Shoji, S., and Nakagawa, S. (1988). Serum prealbumin and retinol-binding protein concentrations in Japanese-type familial amyloid polyneuropathy. *Eur. Neurol.* **28,** 191–193.

Skinner, M., Connors, L. H., Rubinow, A., Libbey, C., Sipe, J. D., and Cohen, A. S. (1985). Lowered prealbumin levels in patients with familial amyloid polyneuropathy (FAP) and their non-affected but at-risk relatives. *Am. J. Med. Sci.* **289,** 17–21.

Westermark, P., Pitkanan, P., Benson, L., Bahlquist, A., Olofsson, B. O., and Cornwell, G. G. (1985). Serum prealbumin and retinol-binding protein in the prealbumin-related senile and familial forms of systemic amyloidosis. *Lab. Invest.* **52,** 314– 318.