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CHANGES IN SERUM AND URINE AMINO ACIDS IN PATIENTS WITH PROGRESSIVE SYSTEMIC SCLEROSIS TREATED WITH 6-AZAURIDINE TRIACETATE

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Abstract—Homocystine, β-alanine and significant elevations of methionine, threonine and histidine were found in the serum of seven patients with progressive systemic sclerosis as a result of 6-azauridine triacetate treatment. Substantial increases in urinary β-alanine, β-aminoisobutyrate and homocystine were also observed. Metabolic changes involving inhibition of pyridoxal phosphate-dependent enzymes are discussed and suggested as a possible mechanism of these metabolic alterations.

THE MAIN biochemical effect of 6-azauridine triacetate (6-AzUrdTA) is inhibition of *de novo* pyrimidine biosynthesis. This effect has been well characterized.[1] Very little attention has been given to the effect of this drug on amino acid metabolism. Administration of 6-AzUrdTA to man and laboratory animals produces hyperaminoaciduria resembling the urine changes of inborn hyper β-alaninemia and homocystinuria.[2,3] Recently we have found corresponding changes in serum amino acids in experimental animals after high doses of this drug.[4]

Six-azauridine triacetate has been used therapeutically in a wide spectrum of diseases[5–10] including severe forms of psoriasis.[11,12] It is important to know whether therapeutic doses of the drug produce the same effect on serum amino acids in man as they do in laboratory animals.[4]

Previous studies have shown increased solubility of dermal collagen in patients with inborn homocystinuria.[13] Thus, increased levels of homocystine caused by 6-AzUrdTA might have a beneficial effect in the treatment of scleroderma. In this paper we present data on changes of serum and urine amino acids in patients with progressive systemic sclerosis, treated with 6-AzUrdTA. This therapy was without clinical improvement in these patients.

METHODS

Seven women with progressive systemic sclerosis, 44–62 years of age, hospitalized at the Clinical Center, National Institutes of Health, were subjects of this study. They

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were given 6-AzUrdTA (Azaribine™)‡ orally for 7-day periods, beginning with 3 g daily and increasing by 3 g daily each period for a total of four periods and a maximum dose of 12 g daily. The dosage of 6-AzUrdTA was 1·75 to 2·70 g/m² at 3 g daily and 7·00 to 10·80 g/m² at 12 g daily. Each daily dose was divided into three equal portions and given at 8-hr intervals. Blood samples were taken at 8.00 a.m. after an overnight fast, before treatment and at the end of each 7-day period 8 hr after the last dose of drug. Twenty-four-hr collections of urine were obtained from all patients before and during 6-AzUrdTA treatment. Amino acids were measured in aliquots of urine and trichloroacetic acid supernatants of serum using standard techniques for analysis of physiologic fluids[14] on a Beckman 120C amino acid analyzer equipped for high sensitivity.

RESULTS

Changes in serum amino acids were found in all seven patients while receiving 9 g daily of 6-AzUrdTA (Table 1). These changes consisted of the appearance of homo-

TABLE 1. SERUM AMINO ACID LEVELS IN SCLERODERMA PATIENTS BEFORE AND DURING TREATMENT WITH 6-AZURDTA*

Amino acid	Pretreatment	Treatment
Taurine	118·7 ± 16·7	122·6 ± 18·0
Aspartic acid	16·1 ± 2·1	18·2 ± 2·4
Threonine	121·6 ± 11·8	196·2 ± 26·3†
Serine	108·6 ± 15·4	108·2 ± 14·6
Glutamic acid	62·9 ± 8·8	71·9 ± 12·6
Glycine	258·4 ± 45·5	397·2 ± 62·3
Alanine	311·2 ± 20·4	345·0 ± 42·5
Valine	148·7 ± 14·7	148·9 ± 13·2
Methionine	20·4 ± 2·7	67·3 ± 14·2†
Isoleucine	52·9 ± 4·5	64·5 ± 14·1
Leucine	107·9 ± 9·3	107·2 ± 8·1
Tyrosine	60·5 ± 3·5	64·9 ± 3·4
Phenylalanine	61·1 ± 5·6	67·2 ± 6·8
β-Alanine	‡	23·7 ± 3·6†
Homocystine	‡	42·3 ± 14·3†
Ornithine	122·6 ± 15·2	181·8 ± 22·5
Lysine	204·1 ± 14·4	254·5 ± 31·5
Histidine	67·9 ± 6·7	120·7 ± 14·7†
Arginine	129·0 ± 18·5	123·8 ± 14·3

* Results are expressed in nanomoles per millilitre of serum and represent the mean ± S. E. M. of one determination in each of seven patients. Treatment values obtained during last day of 6-AzUrdTA administration, 9 g daily. The ranges of amino acid concentrations in plasma of normal persons, as obtained from the literature,[15] are: (nmoles/ml) Taurine 32–138, Asp 1–11, Thr 76–194, Ser 76–164, Glu 20–90, Gly 179–587, Ala 213–472, Val 168–317, Met 11–30, Ile 40–99, Leu 78–176, Tyr 22–83, Phe 38–73, no β-Ala or Homocystine, Ornithine 30–64, Lys 105–207, His 32–97 and Arg 40–140.

† Results are significantly different from pretreatment value with $P < 0{\cdot}05$.

‡ None detectable, i.e. < 2 nmoles/ml.

‡ Generously donated by CalBiochem Company.

cystine and β-alanine and of significantly increased levels of threonine, methionine and histidine. Serum levels of other amino acids such as glutamic acid, glycine, alanine, isoleucine, tyrosine, ornithine and lysine were also apparently increased, but these changes were not statistically significant. Our patients had pretreatment levels of serum amino acids within the ranges reported for normals from the literature for all amino acids but ornithine.[15] The approximate 2-fold increase in the pretreatment level of this amino acid is unexplained.

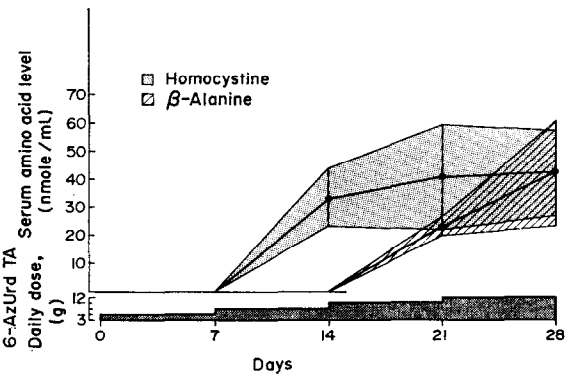

FIG. 1. Serum levels of homocystine and β-alanine during treatment with 6-AzUrdTA. Results are expressed as mean ± S. E. M. of one determination in each of seven patients.

Homocystine was first detected in sera taken during treatment with 6-AzUrdTA at a dose of 6 g daily. Homocystine serum levels reached a maximum with the 9 g dose and remained the same at the 12 g dose (Fig. 1). β-Alanine first appeared in the sera of treated patients at a dose of 9 g daily and increased again as much at a dose of 12 g (Fig. 1). Serum levels of methionine and threonine (Fig. 2) increased with increasing doses of 6-AzUrdTA, reaching maxima at a dose of 9 g daily.

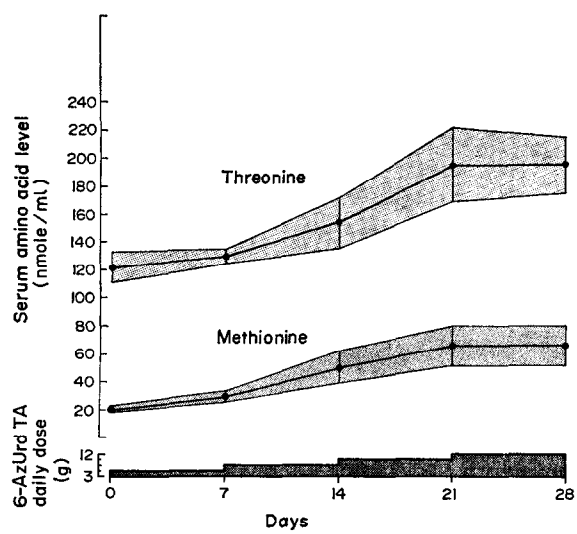

FIG. 2. Serum levels of threonine and methionine during treatment with 6-AzUrdTA. Results are expressed as mean ± S. E. M. of one determination in each of seven patients.

The levels of β-alanine, homocystine and β-aminoisobutyric acid were also measured in the urine (Table 2). β-Alanine and homocystine were undetectable and only traces of β-aminoisobutyric acid were found in pretreatment urines. During 6-AzUrdTA therapy, however, the urinary levels of all three amino acids increased markedly to mean values of 3·41, 0·43 and 0·87 m-moles/day respectively.

TABLE 2. URINE AMINO ACID LEVELS IN SCLERODERMA PATIENTS TREATED WITH 6-AZAURIDINE TRIACETATE*

Pt No.	β-Alanine	β-AIBA	Homocystine
1	3·44	0·98	0·39
2	3·01	1·07	0·02
3	1·90	0·46	0·03
4	5·10	1·16	0·51
5	2·67	0·59	0·81
6	3·82	0·94	0·50
7	3·94	0·91	0·96
Mean ± S. E. M.	3·41 ± 0·38†	0·87 ± 0·09†	0·43 ± 0·13‡

* Results are expressed in millimoles per 24 hr. Values obtained during last day of 6-AzUrdTA administration at either 9 or 12 g dose. β-Alanine and homocystine were undetectable and only unquantifiable traces of β-AIBA were found in pretreatment urines.

† Results are significantly different from pretreatment value with $P < 0·01$.

‡ Results are significantly different from pretreatment value with $P < 0·02$.

While there were no beneficial effects from the 6-AzUrdTA therapy, there were side effects. Nausea, occasional vomiting and anorexia were noted by most patients at both the 9 and 12 g doses. Generalized joint stiffness in the morning, arthralgias, sedation and psychic retardation were reported by some patients at the 12 g dose. A hypochromic anemia was noted in all patients at the 12 g dose. All of these side effects rapidly reverted to normal when the drug was stopped.

DISCUSSION

During 6-AzUrdTA treatment, the unusual amino acids β-alanine and homocystine appear in the blood and there are significant increases of threonine, methionine and histidine. Both β-alanine and homocystine were reported in the urine of patients with 6-AzUrdTA,[2,3] as well as in the serum of treated animals.[4] This is the first evidence that both amino acids are present in the serum of man during 6-AzUrdTA treatment.

β-Alanine is not present in human serum under normal circumstances. Increased serum levels of β-alanine and urinary excretion of β-alanine and β-aminoisobutyrate in man are found in inborn hyper β-alaninemia.[16] β-Alanine and β-aminoisobutyrate are intermediates in the degradation of uracil and thymine. Each of these amino acids is normally metabolized to the corresponding aldehyde (malonaldehyde and methylmalonaldehyde) by a pyridoxal-dependent transaminase.[17]

Appearance of homocystine in blood and urine and of significantly increased levels of methionine in serum of treated patients resembles serum amino acid changes in inborn homocystinuria[18] and suggests blockade of the pyridoxal enzyme, cystathionine synthase. Threonine is an essential amino acid in man[19] and there is no evidence that it can be synthesized in the human body. Thus, significantly increased levels of serum threonine during 6-AzUrdTA treatment could only be explained by inhibition of threonine degradation. The degradation of threonine in mammals is accomplished by two enzymes: threonine dehydrase,[20–22] and hydroxyamino acid aldolase.[23,24] Both of these enzymes are pyridoxal phosphate dependent.[25,26]

Serum histidine levels in scleroderma patients were low before treatment when compared to normals, but almost doubled during 6-AzUrdTA treatment. The major pathway of histidine degradation in mammals is via conversion to urocanic acid which is split by the enzyme urocanase.[19] This latter enzyme is also pyridoxal phosphate dependent.[27]

The changes in plasma and urinary amino acids found after treatment with 6-AzUrdTA appear to be consistent with a general decrease in catabolism. A common feature of all of the amino acid-catabolizing enzymes discussed is the utilization of the cofactor, pyridoxal phosphate. Also one explanation for sedation, psychic retardation and hypochromic anemia as side effects of the 6-AzUrdTA is pyridoxine deficiency. Thus, 6-AzUrdTA or one of its metabolites may be acting as a pyridoxal phosphate inhibitor. This is presently indirect evidence since measurements of pyridoxal phosphate or pyridoxal enzymes have not been made in patients or animals treated with 6-AzUrdTA. This study provides evidence for a unitary hypothesis to explain the amino acid changes noted in patients given 6-AzUrdTA. It also suggests that pyridoxine administration may reverse these metabolic alterations.

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