Serum Kynurenine in Rheumatoid Arthritis

HARRY SPIERA and RAMON VALLARINO

From the Department of Medicine, Mount Sinai School of Medicine of The City University of New York, New York 10029

ABSTRACT The concentration of kynurenine has been measured in the serum of patients with rheumatoid arthritis and in a control group. The mean serum concentration was 2.27 ± 0.688 μg/ml for patients with rheumatoid arthritis and 2.95 ± 0.825 μg/ml for the control group.

Kynurenine: creatinine clearance ratios were higher in the rheumatoid arthritis than in the control group. These data suggest that the increased urinary excretion of kynurenine by patients with rheumatoid arthritis is due at least in part to renal factors.

INTRODUCTION

Most patients with rheumatoid arthritis excrete in their urine increased quantities of metabolic products of tryptophan (1–6). The products most consistently found in abnormal quantities are kynurenine, 3-hydroxyanthranilic acid, hydroxykynurenine, and xanthurenic acid. These are all of the kynurenine pathway of tryptophan metabolism. Products of the serotonin pathway are excreted in normal quantities. On this basis it was postulated that patients with rheumatoid arthritis had increased hepatic tryptophan pyrrolase activity resulting in a shunt of tryptophan into the kynurenine pathway (7). Subsequently, studies performed on liver biopsy specimens obtained from patients with rheumatoid arthritis demonstrated increased tryptophan pyrrolase activity in those patients with increased kynurenine excretion (8). The number of patients studied in this fashion was small, but the results seemed to verify the hypothesis offered to explain the abnormality of tryptophan metabolism manifested by patients with rheumatoid arthritis.

An alternate possibility, that the increased excretion could be due to renal rather than metabolic factors, could not heretofore be tested for lack of suitable techniques for the measurement of these metabolites in serum. We have been able to adapt Thompsett's (9) method for measurement of kynurenine in urine to its measurement in serum by using an absorption cell with a long light path. Using this method we have studied the serum kynurenine concentration in a group of patients with rheumatoid arthritis and in a control group. We have also studied kynurenine: creatinine clearance ratios in these two groups. These studies form the basis of this report.

METHODS

Patients were from the clinics and wards of The Mount Sinai Hospital. Patients with rheumatoid arthritis met the criteria for definite or classical rheumatoid arthritis as defined in the American Rheumatism Association criteria (10). Control patients were healthy employees.

Bloods were drawn in the fasting state and the sera separated and frozen till they were analyzed. Urines were collected in the morning after the initial specimen had been discarded. No drugs or food were allowed till after the urine collection; water intake was encouraged.

Urine kynurenine was determined by the method of Thompsett (9). Serum and urine creatinine were measured with a Technicon AutoAnalyzer by standard methods (11). For studies of clearance ratios the patients were instructed to empty their bladder on arising and consume 250 cc of water. 1 hr later blood was drawn, and 2 hr later the bladder was emptied. Clearance ratios were calculated by the formula: $R = C_{kyn}/C_{cre} = U_{kyn} \times P_{cre}/U_{cre} \times P_{kyn}$.

Serum kynurenine was determined as follows:

Serum kynurenine. Into a 45 ml plastic centrifuge add 8.0 ml of serum and dilute to 10 ml with distilled water, add 10 ml of 20% trichloroacetic acid, stir with two round applicators, wait 5 min, stir again, and centrifuge for 5 min. The supernatant is transferred to a 25 ml graduated cylinder, and the volume is recorded. It is then transferred to a 120 ml sidearm distilling flask and diluted to 40 ml with distilled water. To the above, 10 ml of saturated sodium hydroxide is added. Three boiling chips and a tuft of Pyrex glass wool are placed into the flask. Two additional glass tufts are added in the neck of the distilling flask, one at the base of the neck above the bulb, the other just below the side arm. The flask is stoppered with a No. 3 rubber stopper and mounted in a fluidized sand bath [1] and distilled at 125°C. The distillate passes through a 7¾ inch condenser and col-

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[1] Tecam fluidized bath, Type SBC Techne (Cambridge) Ltd., Duxford, Cambridge, England.

lected in a 25 ml Erlenmeyer flask, and the first 20 ml is collected.

The above 20 ml of distillate is transferred to a 50 ml Erlenmeyer flask to which are added 0.4 ml of 10 N HCl + 2 ml of 0.1% sodium nitrite; wait 5 min, add 2 ml of 0.5% ammonium sulfamate; wait 5 min and add 2 ml of Marshall reagent. (Note flask must be stirred after each of the preceding reagents are added.) After 3½ hr read in Beckman spectrophotometer at 540 λ, with a 100 mm Vycor cylindrical cell (Corning Glass Works, Corning, N. Y.) reading optical density.

Only four samples are developed at one time, as there is a long reading time. The blank is 20 ml of distilled water treated as deproteinized serum.

The standard curve is prepared by adding known quantities of kynurenine to serum aliquots. The standard curve must be made up with plasma or serum.

RESULTS

Table I shows the recovery of known amounts of kynurenine added to serum. It can be seen that recovery is 81–109%. Aspirin, propoxyphene, prednisone, and mycochrysine when added to serum did not interfere with the determination. Two patients with psoriasis who were serving as controls were studied three times a week for a period of 4 wk, and the serum kynurenine remained constant within 15%. Changes in serum kynurenine concentration after meals were variable, sometimes rising and sometimes falling; therefore, only fasting specimens were used for the study.

Tables II and III show serum kynurenine concentrations, serum creatinine concentration, urinary excretion of kynurenine per milligram of creatinine, and clearance ratio of kynurenine to creatinine. Serum kynurenine concentration was 2.27 ±(SD) 0.825 μg/ml for patients with rheumatoid arthritis, and 2.95 ±0.825 μg/ml in the control group. The difference is statistically significant ($P < 0.005$).[2] The rheumatoid arthritis group excreted 0.00705 ±(SD) 0.00691 mg of kynurenine per mg of creatinine, while the control group excreted 0.00178 ± (SD) 0.00190 mg of kynurenine per mg of creatinine. The difference is also significant ($P < 0.01$). The clearance ratio of kynurenine to creatinine was 0.0216 ± (SD) 0.0212 in the rheumatoid arthritis group as compared to 0.0048 ±(SD) 0.0046 in the control group. The difference is significant ($P < 0.01$). Serum creatinine concentrations were similar in both groups.

TABLE I

*Recovery of Kynurenine Added to Serum**

Added	Recovered	Recovered	Final concentration
μg/ml	μg/ml	%	μg/ml
0.25	0.24	97	1.09‡
0.50	0.54	109	1.39‡
0.75	0.71	94	1.56‡
1.00	0.88	88	1.73‡
1.25	1.01	81	1.98§
1.50	1.52	100	2.49§
2.00	1.82	91	2.79§
2.50	2.22	89	3.19§
3.00	2.66	89	3.63§

* Results of two experiments using different sera over the following ranges: 0.25–1.00 μg/ml and 1.25–3.00 μg/ml. The above values are the means of three determinations.

‡ Serum kynurenine concentration without added kynurenine: 0.85 μg/ml.

§ Serum kynurenine concentration without added kynurenine: 0.97 μg/ml.

TABLE II

Rheumatoid Arthritis Group

Patient	Serum kynurenine	Serum creatinine	Urinary excretion: mg kynurenine / mg creatinine × 10⁵	R × 10⁴*
	μg/ml	mg/100 ml		
S. H.	1.95	0.572	474	139
M. E.	1.63	1.080		
S. L.	1.73	1.120		
W. S.	2.46	0.936		
P. F.	2.96	0.789	161	42
D. T.	2.91	0.672	668	48
J. S.	3.45	0.648	260	154
F. S.	1.66	1.120		
D. V.	1.49	0.440	1324	390
M. T.	2.19	0.584	2895	772
B. G.	1.97	0.664	702	236
B. S.	1.23	0.544	408	180
F. G.	1.36	0.744	1162	635
W. W.	3.61	0.880	343	83
M	2.32	0.880	198	75
E. S.	1.62	0.592	353	129
H. S.	3.47	0.800	673	155
M. C.	1.59	0.496	360	112
I. C.	2.42	0.560	1142	264
S. D.	3.36	0.960	149	42

* $\frac{\text{Urinary kynurenine} \times \text{serum creatinine}}{\text{urinary creatinine} \times \text{serum kynurenine}}$.

DISCUSSION

The data clearly demonstrate that the serum concentration of kynurenine is low in approximately 50% of patients with rheumatoid arthritis. The combined urine and serum data show a considerably increased renal clearance of kynurenine by patients with rheumatoid arthritis. It is therefore quite evident that renal factors play an important role in the increased urinary excretion of kynurenine by these patients. On the basis of earlier find-

[2] The probabilities are based on Behren's modification of the Student's t test as used when the variances are significantly unequal.

TABLE III
Control

Patient	Serum kynurenine	Serum creatinine	Urinary excretion: mg kynurenine / mg creatinine × 10^5	R × 10^4*
	μg/ml	*mg/100 ml*		
M. T.	2.90	0.940	142	46
Z. L.	2.45	0.864	55	19
R. V.	3.24	0.920	104	29
C. F.	2.95	0.688	522	121
S. S.	2.66	0.912	125	42
L. T.	2.60	0.968	91	33
P. O. P.	5.48	0.968	117	20
B. J.	2.85	0.800	230	64
M. G.	2.54	0.560	208	45
R. M.	2.76	0.672	1058	257
N	3.06	0.704	244	56
N. J.	2.41	0.848	104	36
P. T.	2.62	0.660		
S. R.	2.61	0.808	158	48
F. F.	3.94		105	
K. J.	2.59	0.760	215	63
C. A.	3.73	0.968	143	37
J. H.	3.35	1.02	104	31
T. A.	2.63	0.776	141	41
B. J.	2.51	1.00	107	42
K. A.	2.27	0.696	158	48
W. K.	5.82	0.712	106	12
B. B.	3.15	0.840	101	26
H. M.	2.70	0.632	93	21
C. M.	2.63	0.840	89	28
G. A.	2.36	0.768	89	28
V. G.	2.35	0.672	227	64
B. K.	2.48	0.792	90	28
F. M.	3.60	0.864	133	31
B. E.	2.34	0.792	107	36
I. F.	2.40	0.720		
L. P.	2.41	0.504		

* $\frac{\text{Urinary kynurenine} \times \text{serum creatinine}}{\text{urinary creatinine} \times \text{serum kynurenine}}$.

ings from this laboratory, it was suggested that the increased excretion of the tryptophan metabolites by patients with rheumatoid arthritis was due to increased production consequent to increased tryptophan pyrrolase activity. We had previously shown that the most effective method of increasing urinary excretion of 3-hydroxyanthranilic acid in the rabbit was the administration of hydrocortisone, a potent inducer of tryptophan pyrrolase (12). Altman and Greengard (8) studied tryptophan pyrrolase activity of liver biopsy specimens. Among them were specimens obtained from patients with rheumatoid arthritis. The patients who excreted increased quantities of kynurenine had increased tryptophan pyrrolase activity. It was therefore concluded that increased tryptophan pyrrolase activity was indeed the mechanism for the abnormal tryptophan metabolism noted in patients with rheumatoid arthritis. The present study casts doubt on this view. It is possible that both renal and metabolic factors play a role. (One may speculate that due to increased renal loss of kynurenine, serum kynurenine is decreased. This loss could decrease feedback inhibition of tryptophan pyrrolase activity, and thus lead to increased tryptophan pyrrolase activity.) In vitro studies of kynurenine have been shown to inhibit tryptophan pyrrolase (13).

No information is at present available as to the serum concentration of hydroxykynurenine, xanthurenic acid, kynurenic acid, 3-hydroxyanthranilic acid, *n*-methylnicotinamide, and pyridone. Studies of renal clearance of these substances await the development of suitable methods for the determination of these substances in serum. The methods of Coppini, Benassi, and Montorsi (14) are unsuitable, as their methods are applicable only when the concentration of these substances has been increased by massive tryptophan loading. Tryptophan itself is a potent inducer of tryptophan pyrrolase (15), and therefore the test itself could result in the increased production and excretion of kynurenine.

The significance of the increased excretion of tryptophan metabolites in rheumatoid arthritis is not known. Neither the urinary excretion nor serum concentration of kynurenine seem to bear any relation to the activity of the disease, nor to any other clinical or laboratory parameter studied. Suggestion that the abnormality is related to absolute or relative pyridoxine deficiency is inadequate to explain the increased excretion of 3-hydroxyanthranilic acid. Pyridoxine is a cofactor for the enzyme kynureninase, and deficiency would result in decreased rather than increased 3-hydroxyanthranilic acid.

Steroids in large doses will induce tryptophan pyrrolase activity and thereby increase kynurenine production (8). However, the dose of steroids used in the management of rheumatoid arthritis is smaller than that necessary for the induction of enzyme. In our experience doses less than 10 mg of prednisone per day do not influence kynurenine excretion.[8]

Disturbances in tryptophan metabolism have also been reported in many other diseases, including among others leukemia, Hodgkin's disease, carcinoma of the bladder, schizophrenia, and scleroderma (16). In most of the studies, the metabolic products were measured only in urine. As shown in the present investigation, it would seem that both urine and serum must be studied before any conclusion can be drawn as to the cause of the abnormality.

[8] Spiera, H. Unpublished observations.

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