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SHORT COMMUNICATIONS

Alterations in metabolism of copper and zinc after administration of 6-azauridine triacetate

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6-AZAURIDINE TRIACETATE (6-AzUrdTa) is an anti-metabolite which inhibits *de novo* pyrimidine biosynthesis.[1] It was originally developed as an anti-tumor agent,[1] and it has been effective in the treatment of several malignant conditions in man.[2–5] However, because of its low toxicity,[6] it has been used in the treatment of several non-neoplastic diseases[6–10] including psoriasis.

In previous reports, administration of this drug to man[11,12] and to experimental animals[11,13] was associated with alterations in amino acid metabolism, including changes in thiol containing amino acids and in histidine. Since the relationship between these amino acids and the metabolism of several metal ions is well known,[14–17] it was of interest to study the changes of copper and zinc in blood and urine of patients before and during 6-AzUrdTa administration in order to describe the manner by which these changes occur.

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 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. Blood was drawn in plastic syringes fitted with stainless steel needles and immediately transferred to metal-free containers. Blood was allowed to clot, centrifuged, the serum removed, transferred to other metal-free containers and stored at −20° until assayed. Twenty-four-hr collections of urine were obtained from all patients before and during 6-AzUrdTa treatment. Urine was collected in plastic containers, under refrigeration, from each patient from 8:00 p.m. to 8:00 p.m. the following day. In this way the 8:00 a.m. blood sample fell midway between each 24-hr period. Amino acids were measured in aliquots of urine and trichloroacetic acid supernatants of serum using standard techniques for analysis of physiologic fluids[18] on a Beckman 120C amino acid analyzer equipped for high sensitivity.

Analysis of total copper and zinc concentrations in serum and urine were carried out simultaneously by atomic absorption spectrophotometry by a method previously described.[19] Serum and urine were also analyzed for creatinine by a method previously described.[20] With these data renal clearances for copper and zinc were calculated for each 24-hr period as the amount of copper or zinc excreted per min per mg of creatinine, assuming that a fraction of both metals is filtered and reabsorbed. All blood and urine samples were coded such that no knowledge of the dose of drug given to the patient was imparted to those individuals performing the analytical procedures.

Administration of graded doses of 6-AzUrdTa resulted in a significant decrease in serum concentrations of total copper ($P > 0.05$, paired t-test) without significant changes in urinary copper excretion (Fig. 1). The decrease in total serum copper concentration was initially measured after administration of 3 g of 6-AzUrdTa for 7 days. With administration of greater amounts of the drug only small further decreases in serum copper were observed. The total serum copper concentration in the untreated patients (142 ± 8 μg/100 ml, mean ± S.E.M.) was significantly higher ($P < 0.001$) than that of normal volunteers (96 ± 2 μg/100 mg[21]). After administration of 6-AzUrdTa, this level decreased to levels that were not significantly different from normal after 28 days of drug administration (118 ± 7 μg/100 ml).

Administration of graded doses of 6-AzUrdTa resulted in a significant decrease in serum concentration of total zinc after 21 days of the study ($P < 0.05$, paired t-test) and a significant increase in urinary zinc excretion (Fig. 2) after 14 days of the study ($P < 0.001$, paired t-test). Total serum zinc concentration decreased after administration of 3 g of 6-AzUrdTa for 7 days, but it decreased significantly only after administration of larger dosages of 6-AzUrdTa. The mean level from which these decreases in serum zinc occurred (87 ± 9 μg/100 ml) was not significantly different from that of normal volunteers (92 ± 2 μg/100 ml). Increases in urinary zinc excretion first occurred only after

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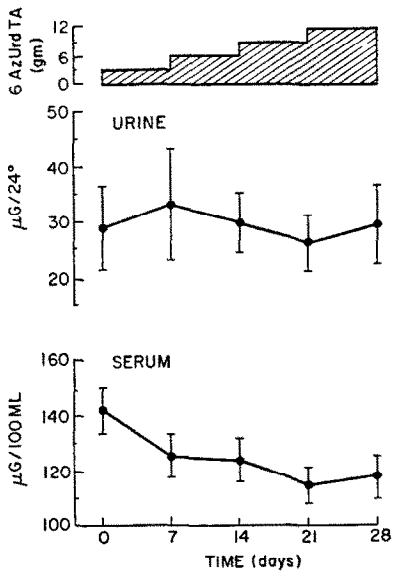

FIG. 1. Changes in total serum copper concentration and in urinary copper excretion during oral administration of 6-AzUrdTa. Serum copper concentration decreased with administration of 6-AzUrdTa. Each point represents the mean ± 1 S.E.M. obtained for seven patients.

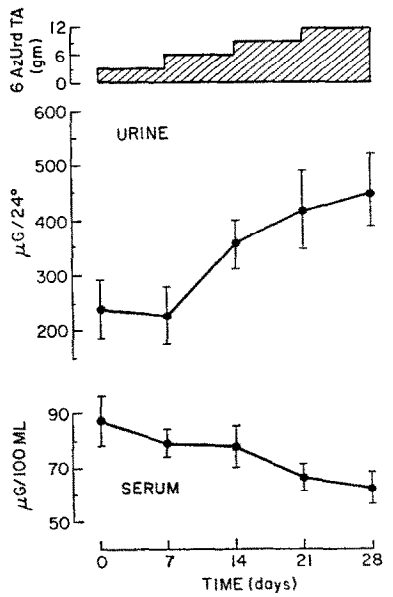

FIG. 2. Changes in total serum zinc concentration and in urinary zinc excretion during oral administration of 6-AzUrdTa. Serum zinc concentration decreased with administration of 6-AzUrdTa while urinary zinc excretion increased.

administration of 6 g of 6-AzUrdTa for 7 days and continued to increase with administration of increasing doses of the drug. Urinary excretion of zinc after administration of 12 g of 6-AzUrdTa was approximately twice the control level of excretion of this metal. The mean level from which these increases occurred in urinary zinc (237 ± 52 μg/24 hr) was significantly lower ($P < 0.05$) than that of normal volunteers (353 ± 23 μg/24 hr[19]) and increased during treatment to levels that were within normal limits (445 ± 70 μg/24 hr).

Expression of the excretion of copper and zinc in terms of renal clearance of both metals does not change the pattern of excretion previously noted (Fig. 3); i.e., after 7 days of 6 g of 6-AzUrdTa there was a significant increase in the renal clearance of zinc while there was no significant change in renal clearance of copper (Fig. 3).

These data demonstrate that administration of 6-AzUrdTa in man results in an increase in urinary zinc excretion and decreases in the serum concentrations of zinc and copper. Changes in serum copper concentration resulted in a decrease in total copper from a level significantly above normal prior to treatment to a level within normal limits after treatment for 28 days. Changes in urinary zinc excretion resulted in an increase in total zinc excretion from a level significantly below normal to within normal limits.

Changes in serum and urinary concentrations of several amino acids have occurred during administration of graded dosages of 6-AzUrdTa.[12] The sulfur containing amino acid homocystine appeared in serum and urine and methionine and histidine increased in serum.[12] It is of interest that the low urinary excretion of zinc in these patients with progressive systemic sclerosis prior to treatment occurred in the presence of a lower than normal serum concentration of histidine.[12]

The observed alterations in zinc and copper metabolism may be related to the hyper-aminoacidemia and hyper-aminoaciduria which occurred during 6-AzUrdTa administration. Increases in histidine and cysteine have been associated with alterations, *in vitro*,[14,15] in the manner by which zinc and copper are bound to albumin and in man with increased excretion of zinc and copper in the urine and with subsequent depletion of both metals from the body.[14,22,23] This same phenomenon may occur *in vitro* or *in vivo* with increased concentrations of homocystine or methionine stripping zinc and copper from their binding sites on albumin. The homocystine–zinc, methionine–zinc complexes

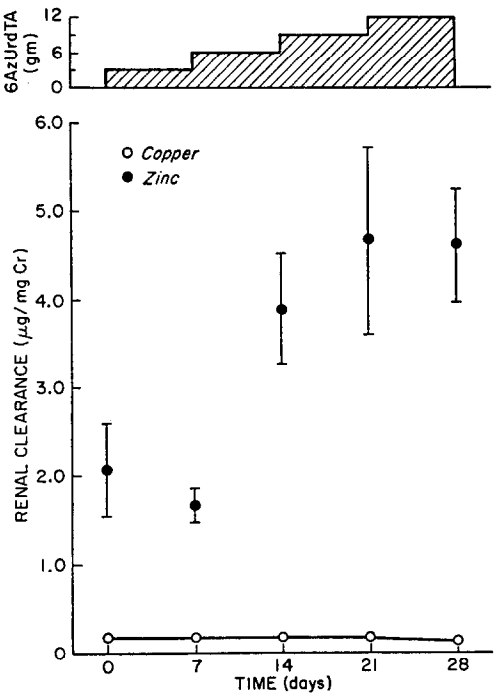

FIG. 3. Renal clearance of copper and zinc during oral administration of 6-AzUrdTa. Renal zinc clearance increased after 6 g of 6-AzUrdTa and remained elevated with administration of increasing doses of the drug. No change in the renal excretion of copper was observed from control levels after any dose of 6-AzUrdTa.

would then pass the renal glomerulus and result in hyperzincuria. Copper is affected less than zinc since its binding to the histidine moiety on albumin *in vivo* is apparently much tighter than that of zinc and thus its binding is not altered to such a great degree. Normally the free amino acid homocystine or histidine are either not present or present in small concentrations in blood or urine. However, after administration of 6-AzUrdTa their concentrations in blood or urine increase significantly and thus play a significant role in altering the metabolism of these metals.

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D-Lysergic acid diethylamide (LSD)—Effect on biogenic amines excretion in man

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Several lines of experimental evidence have advanced the hypothesis that the central action of D-lysergic acid diethylamide (LSD) may involve interaction with serotonergic neurons in the brain.[1,2] Further, it has been shown that LSD may decrease 5-hydroxytryptamine (5-HT) turnover rate,[3–8] antagonize the action of neuronal 5-HT in the raphe nuclei,[9–11] and block 5-HT release from electrically stimulated brain slices.[12,13] The potent pharmacologic effects evoked by microgram doses of LSD have stimulated interest in its application in experimental psychiatry, e.g. as an adjunct for psychoanalytical therapy or as an "experimental model" analogous to naturally occurring mental disorders. However, the relationship of the latter to schizophrenic psychosis is controversial.

Administration of LSD to man induces symptoms indicative of stimulation of the sympathetic nervous system.[14–17] This prompted the quantitative determinations of urinary and plasma catecholamines by several investigators.[18–20] Further, it is likely that central and peripheral serotonergic and/or catecholinergic mechanisms are associated with LSD-induced psychotic-like syndrome in man. Accordingly, the determination of certain biogenic amines which were not previously studied, i.e. 5-HT and dopamine (DA), and their respective major acid metabolites might reflect biochemical changes associated with behavioral manifestation.

A group of seven male psychoanalysts who were in a training program for the use of LSD in psychotherapy volunteered for this study. They provided excellent collaboration for diet control and urine collections. LSD was given by mouth in 200–300 μg at the beginning of psychoanalytical session. Twenty-four-hr urine collections were made for each subject on the day prior to as well as the day of LSD administration. Urine specimens were collected over acid, aliquoted and kept frozen at $-20°$ until assayed. Urine samples were fractionated as previously described.[21] The quantitative determinations of urinary DA, norepinephrine (NE), 5-HT, homovanillic acid (HVA), vanillylmandelic acid